ANATOMY OF THE VEGETATIVE ORGANS OF THE PINEAPPLE, ANANAS COMOSUS (L.) MERR.[1]

I. INTRODUCTION, ORGANOGRAPHY, THE STEM, AND THE LATERAL BRANCH OR AXILLARY BUDS[2, 3]

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Introduction

As research in different phases of pineapple culture has progressed, it has become increasingly evident that some of the problems encountered can finally be solved only through a thorough knowledge of the structure of the plant. From time to time various members of the staff of the Pineapple Research Institute have done some work on the anatomy of certain tissues as the need for immediate knowledge of a specific structure arose. It finally seemed advisable, however, to make a rather complete study of the structure of the entire pineapple plant and at the same time to review and evaluate the already existent literature on the subject. Comparison of the structure of this plant with that of other members of the family Bromeliaceae, as described in the available literature, will also be presented when of interest.

The present paper is a report of a study of the vegetative organs, originally prepared primarily for use by members of the staff of the Pineapple Research Institute and personnel in the pineapple industry in Hawaii. It is by no means an exhaustive study, but it is hoped that, specifically, it will serve as a source of reference for those who need to know some anatomy of the pineapple plant as a background for their own research or work and, generally, contribute to the literature on monocotyledonous anatomy.

There are a great many references to certain structural features of the Bromeliaceae to be found in the general anatomical literature (**1, 7, 30, 31, 34, 38, 40, 41, 42, 46, 47, 49, 50, 53, 54, 56, 57, 58, 59, 75, 76, 79, 80, 81, 83, 90, 100, 103, 104, 109, 113, 116, 117, 118, 119, 120, 127, 136, 142, 147, 155, 159, 163, 164, 165, 168, 170**); although many of these consist of brief descriptions only, in total they represent a considerable contribution to our knowledge of bromeliaceous anatomy. A growing interest in the Bromeliaceae, aroused primarily by SCHIMPER's descriptions of them in their native habitats in tropical America (**118, 119, 120**), and the increasing numbers of bromeliaceous species which became available in the botanical gardens of Europe, stimulated various investigators to make special, fairly detailed anatomical studies of this family. This fundamen-

[1] Interpret Rumph. Herb. Amboin. (1917) 133. This nomenclature for the pineapple is the most widely acceptable at present. It was officially recognized by the Experiment Station of the Association of Hawaiian Pineapple Canners (now the Pineapple Research Institute of Hawaii) in 1933. Other names for this plant seen most frequently in the literature are *Ananas sativus* (Lindl.) Schult. fil.; *Ananassa sativa* Lindl.; and *Bromelia Ananas* L. (**96, 149**). In order to avoid confusion and repetition, only the term *Ananas comosus* will be used in this paper, regardless of the original citation.

[2] Additional sections of this paper will appear in succeeding issues of the BOTANICAL GAZETTE. Literature cited will appear at the end of the last section.

[3] Published with the approval of the Director as Technical Paper no. 186 of the Pineapple Research Institute of Hawaii, Honolulu, T. H.

tal work was done during the relatively concentrated period covered by the last quarter of the past, and the first decade of the present, century by a number of Austro-German and Scandinavian investigators (12, 16, 22, 61, 64, 69, 88, 94, 95, 110, 143, 150, 156, 167). Several of these authors (64, 94, 167), their contemporaries in other countries (14, 15, 106, 112, 161, 162), and others who followed (97, 107, 126, 141) have presented more or less detailed descriptions of all or several of the vegetative organs, while BORESCH (16) described the structure of both the stem and the root. Some papers have been largely concerned with root origin and structure only (69, 93, 157, 158); of these, a recent publication by MEYER (93) is an especially valuable contribution to the literature on bromeliaceous anatomy. SOLEREDER and MEYER (141) have described all the vegetative organs in some detail, reporting on many species of Bromeliaceae.

Probably because of the many singular features characteristic of the leaves of bromeliaceous plants, the greatest number of studies have been made on the structure of these organs. KEILINE (72) and SOLEREDER and MEYER (141) presented descriptions of all the leaf tissues in considerable detail. Other papers, although describing the leaf as a whole rather briefly, describe certain tissues and their functions in some detail: the epidermis (12, 20, 22, 88, 110, 148); the aerating system (22, 88, 148); the assimilatory tissue (110, 148); the water-storage tissue (22, 148); the vascular and mechanical systems (110, 126, 156); and the scutiform trichomes (6, 19, 23, 32, 61, 95, 150, 160).

The number and scope of studies dealing specifically with the anatomy of the pineapple plant are somewhat limited; they include very simple and brief descriptions of the plant, primarily of the fruit, by early botanists and growers, and the following more recent work, presented in chronological order: a brief description of leaf structure (166); a more detailed one of the internal structure of the vegetative organs (65); a brief description of the stomata (21) and of the stem (130); an uncompleted and unpublished, but fairly extensive and detailed, report of the anatomy of the mature plant (13); a study of the structure of the seedling and young plant (98); brief descriptions of the plant as a whole (24, 68); and a report on the origin and development of the leaves (73). Specific references to certain anatomical features of this species are to be found in some of the general anatomical and bromeliaceous literature listed above (16, 22, 23, 30, 72, 88, 93, 110, 126, 141, 154, 167).

More detailed reference to most of the literature cited above will be made in the discussion on the structure of the various organs.

The variety of pineapple principally grown on a commercial scale in Hawaii is the Smooth Cayenne. Material used for the present study was of this variety, from plants of various ages previous to floral differentiation, except in the case where seedling structure was studied; for the latter study hybrid seedlings containing at least 50% Smooth Cayenne were used. The seedlings were grown in propagating flats, under glass. Other young, but not seedling, plant material was obtained from rooted shoot buds grown from Smooth Cayenne stem sections (for details on the propagation of these see SIU [138]). The more mature material was obtained from plants grown in the Wahiawa district, in areas where established plantation practices are employed, in order to have as "normal," i.e.

average, and uniform material as possible.

Observations, drawings, and photomicrographs were made on and from sections of both fresh material and material prepared by usual or modified microtechnical methods. Most of the drawings were made with the aid of a Zeiss camera lucida. Photomicrographs were made with a Bausch and Lomb photomicrographic camera (type K) used in conjunction with Zeiss and Leitz microscopes. Specific information on the preparation of material is given whenever it is considered to be of interest.

The plant:[4] organography

The pineapple plant is a perennial, herbaceous monocotyledon. It stands from 90 to 100 cm. above the ground and has a maximum spread of 130–150 cm.[5] The aerial portion of the stem is completely covered by closely overlapping sessile spirally arranged leaves which hide it completely from view (figs. 1, 2). The leaves form a dense rosette, the outline of which, in longitudinal section, is roughly heart-shaped (fig. 3). The underground portion of the stem is covered with a cluster of fibrous roots (figs. 2, 4, 5).

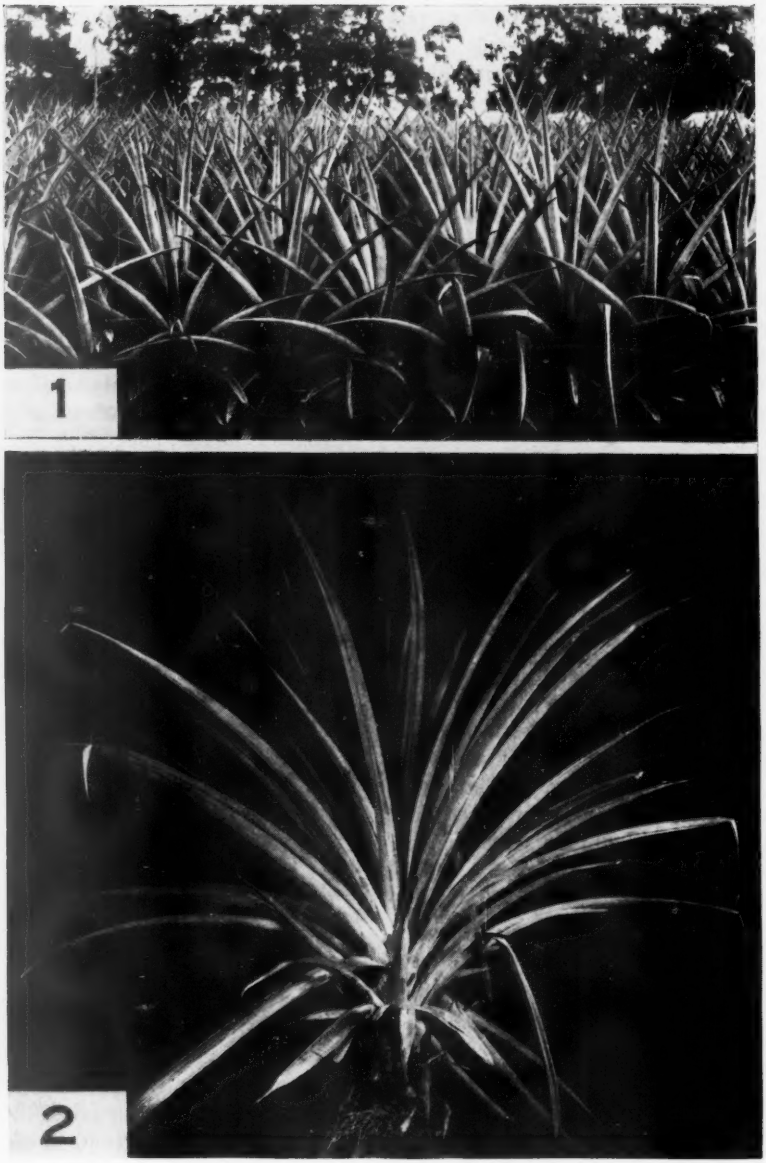
The leaves number from seventy to eighty, exclusive of the dry, wholly dead, basal leaves and the center leaves less than a centimeter in length. They are ensiform, semirigid, and channeled, i.e., concave on the upper side, except at the tip; they are thus crescent-shaped in transverse section (fig. 6*A*). This is said to be typical for the leaves of most Bromeliaceae (**22**). The longest leaves in the rosette (figs. 2, 3, 7) are from 80 to 100 cm. long and about 6 cm. broad at the widest portion above the basal region, about halfway between the base and tip. After reaching the above maximum width, the leaf tapers gradually to a sharply pointed indurated tip, on the margins of which there are usually small deltoid-cuspidate hooked spines (fig. 6*C*). The bases of the fully developed leaves are broad and encircle the stem in a sheathlike manner, covering a lateral distance of approximately two-thirds of the stem circumference. There are often groups of spines on the margins of the leaf base and even on the blade proper. Water, soil particles, and organic materials such as fragments of decaying leaves and dead insects collect in the space between the leaf base and stem. A living fauna and flora are usually found in these leaf axils. Such populations, consisting of many species, have been described as inhabiting many members of the Bromeliaceae in their natural habitats (**106, 107, 118, 119, 120**).

The under, i.e., dorsal or abaxial,[6] side of the leaf appears striated, owing to the occurrence of longitudinal furrows and ridges, and is covered with a silvery white furfuraceous layer. This is often but erroneously called "bloom"; in reality, it consists of the air-filled "heads," i.e., the expanded portion, of the trichomes; these latter are considered one of the most characteristic features of the Bromeliaceae. The upper, i.e., ventral or

[4] The description presented is of a typical plant 14–15 months after planting from a slip, just before floral differentiation. COLLINS (**24**) has previously presented a brief description of a mature, i.e., fruiting, plant of "average type."

[5] Measurements for the size of plants and for the number and size of plant parts presented here and later are average ranges for field-grown plants (see Introduction), based on original data from various sources (**74, 132**), including those obtained by the writer especially for this paper. Data were obtained from plants grown from slips planted during the late summer or fall, this being the more usual time for planting in Hawaii.

[6] The more common adjective, "under," for this side of the leaf will be used in this paper.



FIGS. 1–2.—The pineapple plant. Fig. 1, general view of growing plants in plantation field. One year old. Fig. 2, individual plant just before floral differentiation, i.e., 14 or 15 months after planting of slip, to show dense rosette formed by closely overlapping sessile leaves. Portion only of root system remains attached to base of stem. About 1/9 natural size.

adaxial,[7] surface of the leaf appears glabrous, with color varying from a pea-green to a dark olive-green, depending upon the age of the leaf and the nutrition of the plant. There is also usually a suffusion of red, owing to the presence of anthocyanin, in a broad central streak along the length of the exposed portions of the upper surface of the older leaves.

The shape and dimensions of the stem, considering both the aerial and the subterranean portions, become apparent only after the removal of the soil roots and leaves (figs. 4, 5). In form the stem is clavate; it is about 20–25 cm. long and has a diameter of 2–3.5 cm. at the base and of 5.5–6.5 cm. at the thickest portion, just below the apex. The major portion has approximately this latter diameter (figs. 4, 5). Although the axis of the aerial portion of the stem stands vertically or nearly so in relation to the ground—the stem being negatively geotropic—the basal portion is usually curved. This lower portion is the stem of the original propagating material, i.e., the "slip" or "shoot,"[8] which is curved as a result of its axillary growth on the mother plant. When the crown or top—the leafy terminus of the main axis extending beyond the fruit—is used as propagating material, the basal portion of the stem is not curved.

The stem is marked off externally by poorly defined nodes—these are superficially indicated by the leaf "scars" left after stripping—and by short internodes (figs. 4, 8). A stem with long internodes seldom occurs in this family (**22**). The length of the internodes in the pineapple stem varies from 1 to 10 mm. The shorter internodes are located in those portions of the stem formed during periods of slower and of incomplete growth, i.e., at the base and apex, respectively; and the longer internodes, in the central portion of the stem which is formed during the period of more rapid growth (fig. 4). Similar observations have been previously reported (**13, 130**). The shortness of the internodes accounts for the overlapping and complete covering of the stem by leaves and the consequent rosette form of the leaf cluster.

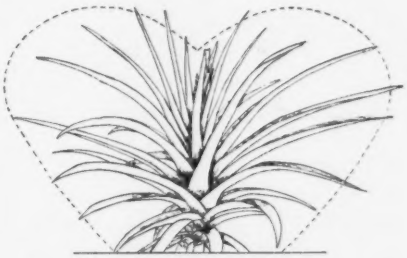
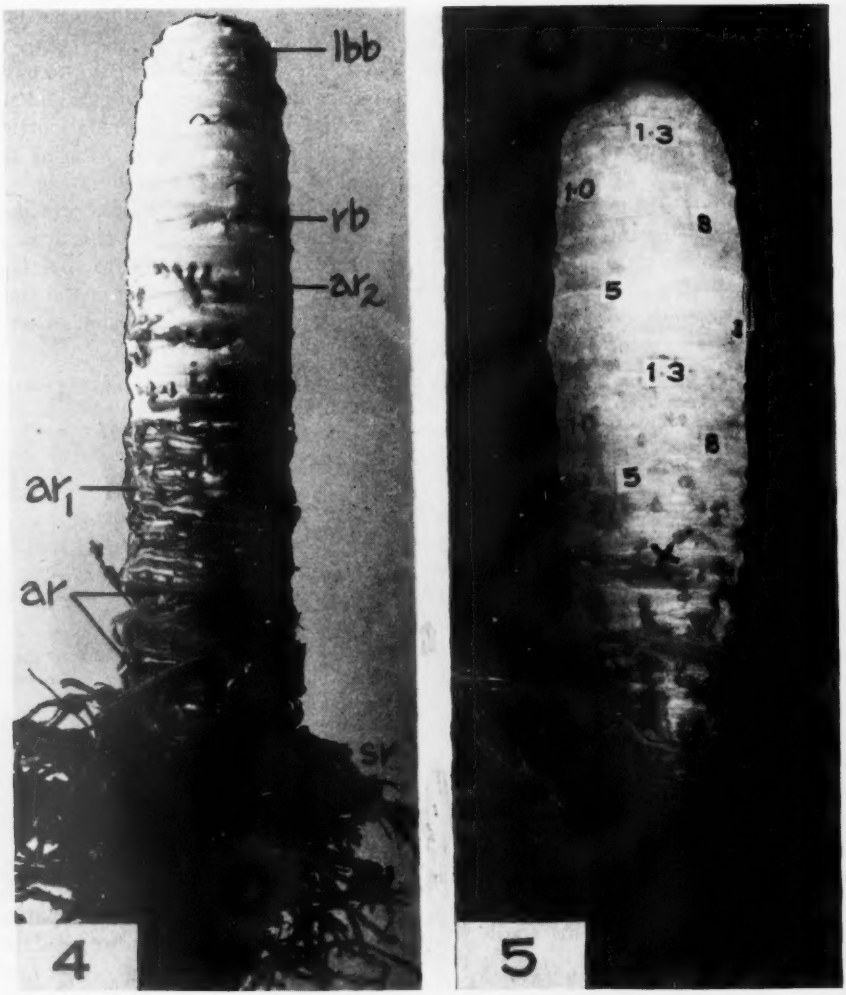


FIG. 3.—Plant showing approximate heart shape of longitudinal section (*broken line*) of leaf rosette. About 1/20 natural size.

The removal of consecutive leaves by stripping makes evident that the leaves are arranged spirally on the stem. Examination of the Smooth Cayenne variety shows that the leaves are arranged in either a right- or a left-hand spiral (**73**), although the former type predominates. The phyllotaxy of the pineapple plant has been established as being $\frac{5}{13}$ (**73, 130**, and SIDERIS and KRAUSS, unpublished data); by stripping leaves from stems and marking the exposed axillary buds which indicate the midpoint of each leaf on the stem, it was found, in following a leaf cycle, that there are five complete turns about the axis and that thirteen of these buds are included in such a cycle (fig. 5). No confirmation for MILES-THOMAS and HOLMES' report (**98**) of a phyllotaxy of $\frac{11}{18}$ in seedling pineapple

[7] The more common adjective, "upper," for this side of the leaf will be used in this paper.

[8] A "slip" is a lateral branch on the inflorescence stem or peduncle. A "shoot" is a lateral branch on that portion of the main stem which is above the ground (see p. 217).



FIGS. 4–5.—Stem. Fig. 4, from 14-month-old plant, with leaves removed to show shape and axillary buds and roots. *sr*, soil roots; *ar*, lower axillary roots which eventually enter soil and become part of soil root system; ar_1, higher axillary roots which never enter soil, remaining tightly wound around stem; ar_2, recently emerged axillary roots higher on stem; *rb*, so-called root "buds" or "primordia" which are actually tips of emerging roots; *lbb*, lateral branch or shoot bud. Approximately 1/2 natural size. (Photograph by R. J. BAKER.) Fig. 5, stem from 12-month-old plant with leaves removed and axillary buds (indicating mid point of leaf "attachment") marked and numbered in sequence to show phyllotaxy. Starting with bud marked *X*, it is shown that complete cycle to another bud directly above this first-marked bud includes thirteen buds arranged in spiral which covers five complete turns about axis. Phyllotaxy is therefore $\frac{5}{13}$. Buds in two complete cycles have been numbered. About 1/2 natural size.

plants, based on an angle of divergence of 220°, was found by these investigators.

Upon removal of the leaves from the stem, it becomes evident that they are not uniform in shape, as it appears from a superficial examination of the intact plant, but that the leaf outline varies with the position of the leaf on the stem. It is principally the shape of the leaf base which differs (fig. 7).

The total number of leaves can be divided, roughly, into two groups, according to development and general form (fig. 7). The first forty leaves—the lower half of the total number—are fully developed and have the general shape represented by the leaves in subgroups 1–3 (fig. 7). The main portion of the leaf—the blade or lamina proper—is lanceolate in form, while the base is considerably expanded. There is a distinct narrowing in width between the basal and main portions of the leaves in this group. This narrow region, which is also a transition zone between the nonchlorophyllous tissue of the base and the chlorophyllous tissue of the blade, has been aptly named the "neck" of the leaf (**131, 132**). The size, especially the length, of these leaves increases with successive, later-formed leaves.

The leaves of this lower group form three fairly distinct subgroups (fig. 7). From the point of view of development the very basal or oldest leaves are the original, already fully developed leaves of the propagating material, the slip or shoot, and therefore change in neither shape nor size after planting. A leaf representative of this subgroup, 1, is shown in figure 7. As the stem elongates and new leaves are formed after the propagating material has been set in the soil, these lowermost leaves become senile and gradually dry back from the tips. At the time of floral differentiation many of them are completely dried and often partially or wholly decayed.

The other leaves making up the lower half of the total number (fig. 7, subgroups 2 and 3) are the originally immature, central leaves of the slip or shoot rosette at the time of planting; these change both in form and in size as they develop after planting.

The older of these leaves (subgroup 2 in fig. 7) are characterized by constrictions in the width of the blade (fig. 6*B*), the zone of constriction varying, in relation to the distance from the proximal or distal ends, with the serial position of the leaf on the stem (fig. 7). This constriction should not be confused with the forementioned neck of the leaf. The portion of the leaf between the constriction and the proximal end represents new growth since planting.

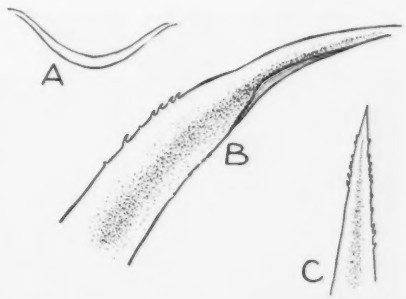


FIG. 6.—Leaf. *A*, transverse section showing crescent shape; *B*, portion to show constriction at boundary between tissue formed in original planting material before (acroscopic), and new tissue formed after (basiscopic), planting; also, marginal spines basiscopic to constriction; stippling indicates central streak of dark red from anthocyanin in subdermal cells; *C*, leaf tip with marginal spines common to Smooth Cayenne variety. About 1/2 natural size.

This group of leaves is also further characterized by the presence of a group of marginal spines in a position basiscopic to the constriction—between the constriction and the leaf base—from 1 to 3 cm. "below" the constriction (fig. 6*B*).

Observations made in various nutritional and drought experiments—carried on by the Physiology-Soils Department (unpublished data) and in root-failure experiments and surveys made by the Pathology Department (**87**) of the Pineapple Research Institute—indicate that adverse conditions, such as nutritional disorders and drought, induce similar groups of marginal spines.

COLLINS recently pointed out (**26**) that the action of the gene for the development of the spiny tip characteristic for the Smooth Cayenne variety appears to be associated with the physiological conditions which initiate leaf growth. Evidently, after the growth processes are under way, the action of this gene for spine production is inhibited; therefore, only the tip of each leaf is provided with

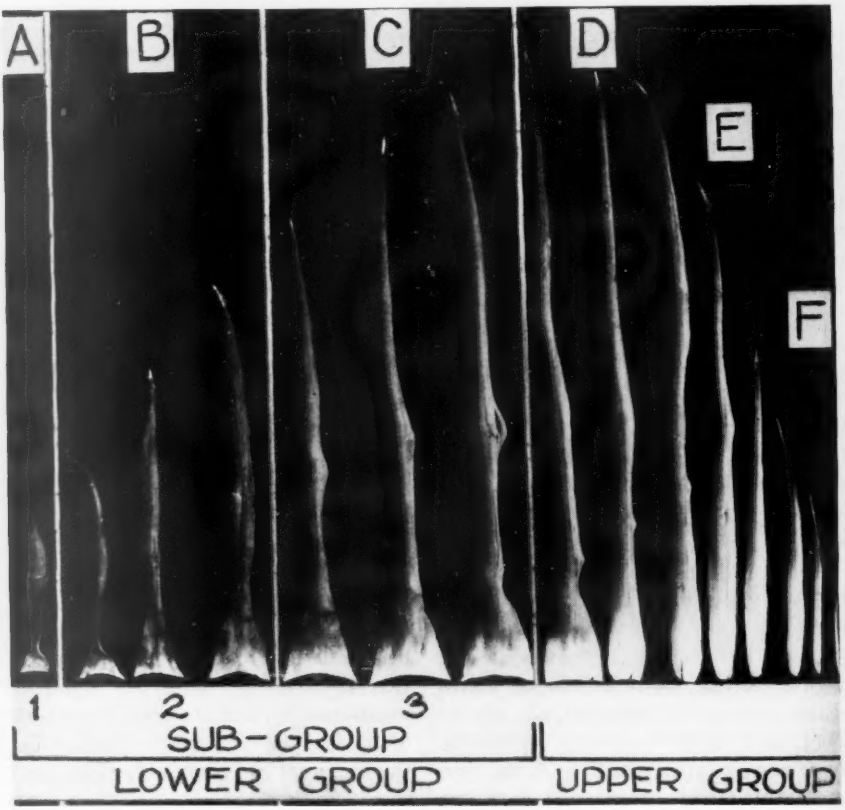


FIG. 7.—Leaf types. Representative leaves from average plant just before floral differentiation, i.e., 14 or 15 months after planting of slip, to show variation in size and shape. Leaves were removed from stem in sequence, beginning at base of stem with oldest nondecayed leaf; representative selections made from each morphological group. Main and subgroups indicated by numbers (pp. 165–167 for description). Letters indicate grouping by SIDERIS, KRAUSS, and YOUNG (p. 167, n. 9). About 1/6 natural size. (Photograph by C. P. SIDERIS.)

spines (fig. 6C). When the propagating material—the slip, shoot, or crown—is removed from a mother plant and left to dry for a time before planting, as is customary in pineapple propagation, leaf growth ceases to all external appearances. This propagating material, after planting in the soil, resumes growth when conditions are favorable. At or near the point on the leaves where new growth starts after this period of growth cessation, indicated by the constriction mentioned above, spines are again produced for a short period of time, and then the inhibiting action comes into play again, resulting in a smooth margin thereafter. The same phenomenon may also occur during any period in the life of the plant in the soil if growth ceases, owing to drought or some other adverse environmental factor, and is then later resumed.

In the remaining subgroup of the leaves which make up the lower half of the total number (subgroup 3 in fig. 7), the leaves show no constrictions; practically the entire growth of these leaves occurs after the propagating material has been planted.

The leaves constituting the upper half of the total number are formed after the planting of the propagating material and, at the stage of plant development being described, exhibit various stages of development. This group consists of the longest leaves of the plant and the shorter central leaves of the rosette. The form of the blade of the longest leaves resembles that of the older leaves already described, but the base is without the acutate expansions, i.e., the "lateral points" (**132, 133**) mentioned as characteristic for the first group of leaves. These leaves are represented by the first leaf in the upper group in figure 7.

The central, youngest leaves of the rosette are lanceolate in form, with no expansion of width into a distinct base; in fact, the width of the proximal portion of these leaves is equal to or less than the width of the broadest portion. These central leaves are represented by the remaining leaves shown in the upper group in figure 7.[9]

The underground portion of the stem is covered with a cluster of fibrous roots, the so-called "soil root system" (**25**). The roots comprising this system have also, at various times, been called basal and

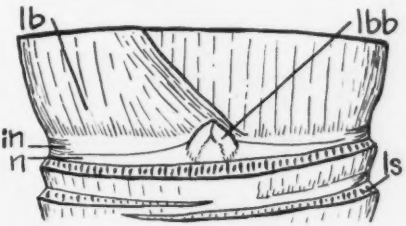


FIG. 8.—Section of stem to show nodal and internodal regions, bud of lateral branch, leaf base "attachment," and leaf scars. *n*, nodal, and *in*, internodal, regions; *lb*, leaf base; *lbb*, lateral branch bud; *ls*, leaf scar. Slightly smaller than natural size.

axillary soil roots (**24, 29**). These roots are adventitious in origin, as a primary root develops only in the seedling and, even there, does not persist for long. Others (**13, 25, 98**) have previously observed this in the pineapple seedling. This condition is apparently characteristic for the Bromeliaceae, since MEZ (**94**) had also observed it in the seedling

[9] Early (**132, 133**) in the research work of the Physiology Department of the Pineapple Research Institute, it became evident that the leaves of the pineapple plant could be separated into consecutive groups, according to morphological characteristics and corresponding stages of development or age; chemical analyses of leaves so segregated showed that these morphological and age groups also represent leaves correspondingly different from a physiological point of view. The grouping of leaves by the present writer corresponds, approximately, to that of the previous writers (groups *A–F* in fig. 7).

representatives of species in five genera of Bromeliaceae which he studied: *Hechtia*, *Puya*, *Deuterocohnia*, *Bromelia* (in which genus he included the pineapple), and *Hepetis*.

According to former established field practice, still followed in certain localities, the slips or shoots are prepared for planting by first stripping off a number of the basal leaves. This operation exposes several "rings," i.e., whorls of root "buds," as they have been called (**17, 68, 69**). Also called "root primordia," these are actually the root tips which are breaking or have broken through the stem epidermis (figs. 4, 9); the true root primordium is, of course, found within the stem, at the point of initiation: the

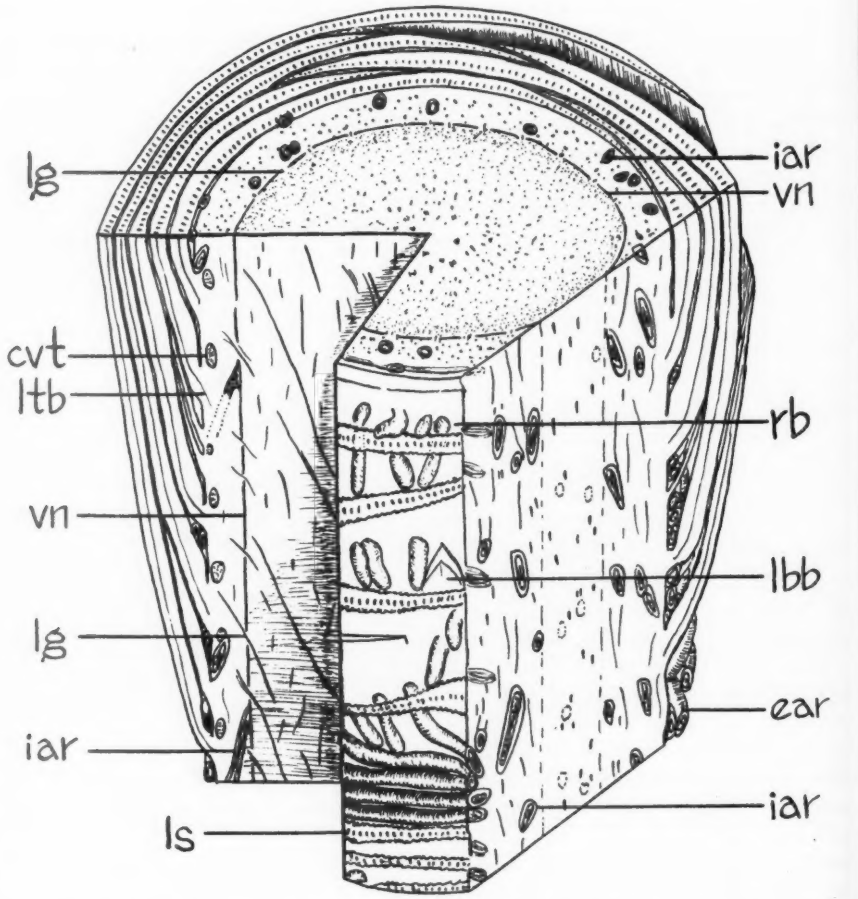


FIG. 9.—Central region of stem cut to show transverse, radial, and tangential views of gross external and internal structure. Tangential section cut at boundary between cortex and stele. *iar*, internal, and *ear*, external, portions of adventitious roots; *cvt*, circumferential band of vascular tissue; *vn*, vascular network at boundary between cortex and steel; *lg*, leaf gap; *ltb*, leaf trace bundle; *ls*, leaf scar; *lbb*, lateral branch bud; *rb*, tip of emerging root, often called root "bud." Slightly larger than natural size.

boundary between the cortex and stele (figs. 9, 10). These exposed root tips swell and elongate, in the presence of moisture, to form the original soil root system. Later, as basal leaves die and decay, roots which develop from consecutive "higher" whorls of root "buds" are added to the first-formed soil root system.

Often the roots emerging at the higher levels elongate to a considerable length and remain confined within the axils of

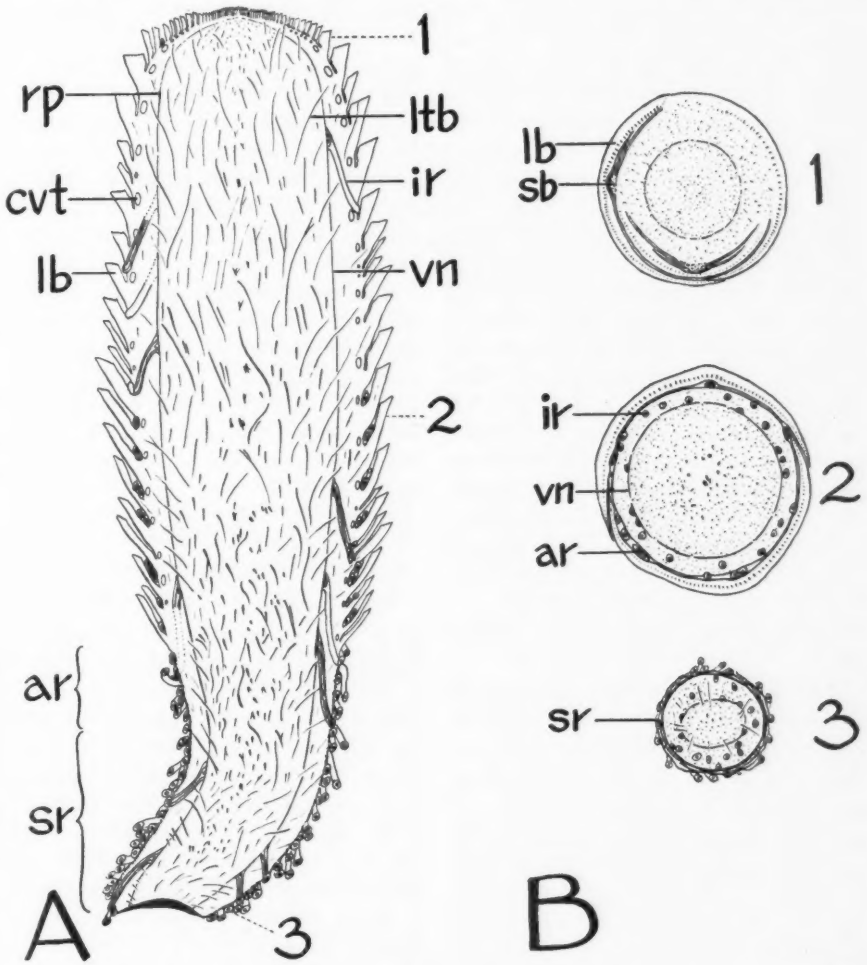


FIG. 10.—Gross internal structure of stem. *A*, radial section of stem just before floral differentiation, i.e., 14–15 months after setting slip in ground, from which soil roots and leaves have been removed. Levels 1, 2, and 3 indicate levels at which transverse sections illustrated in *B* were cut. *ar*, axillary, and *sr*, soil, roots; *lb*, leaf base; *ltb*, leaf trace bundle; *ir*, adventitious root passing through cortex and just emerging at surface of stem; *rp*, root primordium; *vn*, vascular network at boundary of cortex and stele; *cvt*, circumferential band of vascular tissue. About 1/2 natural size.

the leaves before they pass out between the margins of two leaves, or through the bases of leaves which decay, into the soil. These roots, during the period in which they develop in the leaf axils, and those roots which emerge from the stem at higher levels and never enter the soil, constitute the so-called "axillary" root system, as opposed to the "soil" root system (**17, 24, 29, 85, 86**). The axillary roots which never enter the soil are exposed upon stripping the leaves from the stem (figs. 4, 9, 10); they are elongated from a few millimeters in the region near the apex to 10 cm. or more nearer the base of the stem, in the latter case being tightly wound about the stem (figs. 4, 9). They are greatly flattened and distorted as a result of their constricted development between the bases of the leaves and the stem. Although few laterals arise from these axillary roots, they do occur in *Ananas comosus*, contrary to BOWER's statement (**17**) that secondary roots do not develop on axillary roots. JÖRGENSEN (**69**) had previously found in other Bromeliaceae that lateral roots are formed in roots after they have emerged from the stem but before they enter the soil. Root hairs also occur on the axillary roots.

The extent of the soil root system varies with the nutrition of the plant and properties of and conditions within the soil, such as the texture and moisture content, and the presence or absence of parasitic organisms; but, in general, the pineapple plant is considered to be shallow-rooted under average field conditions. For the most part, the root spread laterally is not very extensive (**68**), the penetration not very deep, and the life of the individual root short. Under ideal conditions, however, the soil root system may have a lateral spread of 1–2 meters and penetrate to a depth of 30–85 cm. (**17, 65**). The pineapple soil root branches freely, and secondary, tertiary, etc., roots as well as root hairs are evident.

Also exposed only upon stripping of the leaves from the stem are the shoot buds; these occur singly in the axils of the leaves at the midpoint of each leaf on the stem (figs. 4, 8, 9) and are greatly flattened as a result of being closely appressed between the bases of the leaves and the stem. Most of them are ovate in form, with an acute apex. The buds range in size from 3 mm. in length (i.e., height) and 5 mm. wide at the base of the bud, on the apical and basal portions of the stem, to 2 cm. long and 1 cm. wide on the central portion.

The older portions of both soil and axillary roots, as well as the internodes of the basal portion of the stem and the sheaths (prophylls) inclosing the larger axillary buds, are covered with reddish-brown-colored layers; the color indicates that these layers are composed of suberized cells.

The stem

GROSS INTERNAL STRUCTURE

Examination of radial and transverse sections of a stem (fig. 10*A*, *B*) with the unaided eye shows that the stem, except at the apex, is distinctly divided into a central core and a surrounding ensheathing layer limited externally by the epidermis. Although the monocotyledonous stem is not generally differentiated into a distinct stele, or central cylinder, and cortex, in the sense that these words are used in describing dicotyledonous stems, it seems entirely proper to use these terms in connection with the stem of the pineapple plant. FALKENBERG (**40**) felt justification for the use of these terms in describing many monocotyledons. In descriptions of bromeliaceous plants in general, he and others (**16, 94**) wrote of a

cortex and a central cylinder of the stem, while JÖRGENSEN (**69**) used the terms "outer zone" and "inner zone," respectively, for these tissues. Some authors (**13, 133, 134, 135**) have incorrectly substituted the term "pith" for "stele."

The boundary between stele and cortex is marked by a layer, in the form of a hollow cylinder, which, in the less mature portions of the stem, appears as a conspicuous translucent ring in transverse section or similarly conspicuous lines in radial section (figs. 9, 10); this boundary zone appears in sharp contrast to the remaining farinaceous ground tissue. BORESCH (**16**) described this "ring" as a characteristic feature of bromeliaceous stems. The "dividing cylinder," which consists of a meristematic zone in the younger, and of vascular tissue in the older, portions of the stem, does not appear continuous but is broken or interrupted by openings through which leaf-trace bundles pass (figs. 9, 10). These openings, although not exactly comparable with those in a dicotyledonous stem, may be considered leaf gaps. At the base of the stem the thickness of the layer forming this dividing cylinder is considerably greater, reaching a maximum of perhaps $\frac{1}{3}$ mm., and is often colored a reddish brown, indicating a suberization of its elements. At the apex of the stem this cylinder passes over into a conical sheath, the apex of which seems to be lost at the very tip of the stem (fig. 10*A*). Several investigators (**16, 71, 101, 123, 145**) have described a conical (meristematic) dome in monocotyledons. BORESCH (**16**) reported the occurrence of such an apical meristematic dome in all the Bromeliaceae he studied; he described its presence in *Ananas comosus*, as did BERGMAN and WELLER (**13**).

An examination of radial, especially, and of transverse sections of the stem gives some evidence of the complexity of the vascular system (figs. 9, 10). The view exhibited in a radial section appears a disordered one owing to the diverse and twisted course of the bundles; this has already been commented on by other investigators (**16, 94**). It is, however, evident even at first glance, and especially apparent in transverse sections (fig. 10*B*), that the vascular bundles are distributed throughout the axis, a condition characteristic for monocotyledons in general. Bundles are very numerous throughout the stem but less so in the cortex than in the stele. Transverse stem sections especially show that the central bundles are largest, often appearing branched, and are more widely spaced, while the bundles at the periphery of the stele are smaller and more closely aggregated (fig. 10). There is no evidence of an orientation of bundles into nodal and internodal regions.

In a radial section the bundles are, for the most part, cut longitudinally, although the innermost of the stelar bundles are usually cut obliquely and even transversely. The vascular strands at the apex of the stem are also cut obliquely in radial sections. It is also apparent that only a portion of each bundle is cut or exposed in any single section, which indicates that the course of the bundles is not in a single plane (fig. 10*A*).

The course of the vascular bundles may be studied by several means. Perhaps the easiest method is with retted stems. Longitudinal halves of a stem are allowed to lie in water until such time as bacterial action has destroyed the cellulose structures and thus isolated the lignified and suberized elements. This same operation exposes the gross structure of the older portions of the cylinder dividing the cortex and stele.

When thick longitudinal sections of

the stem are boiled in a heavy sugar solution, they become translucent. The bundles may then be traced with or without staining. Another method of making thick sections translucent is to treat them with a chloral hydrate solution (eight parts of the hydrate and five parts of water). The reaction can be speeded up by heating the sections in the solution at about 50° C. After thorough washing in water, the bundles are best traced when stained with phloroglucin.

Individual bundles were also traced by dissecting untreated stems or, to facilitate the operation, stems which had previously been subjected to vital staining. In the latter case, several centimeters of stem were cut off and the base of the plant placed in a dye, such as fast green. Within 48–96 hours the dye was to be found in many of the bundles of stem and leaves. Another method used in tracing individual isolated groups of bundles was as follows: all absorbing roots but one were cut from the stems of young plants, the severed ends of all roots covered with wax, and the remaining attached root placed in a dye such as fast green.

Examination of the retted stem, especially, shows that all bundles are common bundles, i.e., they pass through the stem for a greater or less distance and continue as leaf-trace bundles. When bundles are followed from the leaves into the stem, many are observed to enter the latter at an angle, usually about 45°, after which they ordinarily penetrate deeply. The larger median bundles usually penetrate more deeply than the smaller lateral ones. As a rule, the larger bundles pass through the cortex into the stele through the leaf gaps previously mentioned and continue, still obliquely, to the center of the stem axis before turning outward and downward in a broad curve toward the periphery of the stele. As these bundles approach the boundary between stele and cortex, they continue for some distance downward, almost parallel to it, before actually approaching and appearing to unite at their lower ends with elements in the vascular network forming this boundary. The downward course of these bundles is never vertical except for this usually short distance, nor is their course limited to a single vertical or lateral plane; in other words, the course is a twisted one within the directions described above. Each common bundle sooner or later fuses or unites with others, the anastomoses occurring frequently and at any point along the course of any one bundle. Several investigators (**13, 16, 30, 40, 155**) have described in more or less detail this characteristic course of bundles in the stems of bromeliaceous plants, including *Ananas comosus* (**13, 30, 155**); this type of bundle-course system represents a modification of the palm type, first described by VON MOHL (**101**).

Although in some monocotyledons anastomoses occur chiefly at, or are restricted to, the nodal region, this is not true in the pineapple. As seen in both radial and transverse sections, the large bundles seem to branch chiefly in the central region of the stem. Viewed from a lower or higher level, it appears as if a bundle abruptly splits into three or more branches; actually, several bundles anastomose with one another at or very near the same level. FALKENBERG (**40**) considered this "vascular bundle branching" to be especially characteristic for the Bromeliaceae, and others (**13, 16, 155**) have observed such anastomosing in the stems of various members of this family, including *Ananas comosus* (**13**).

When the smaller bundles are traced from the leaves into the stem, they also

are observed to enter at an angle, but many of them follow a different course from that of the larger bundles. Many of these smaller bundles are lateral leaf bundles, while others are the smaller bundles which alternate with the larger bundles in the leaf. Upon entering the stem, many of these smaller bundles pass through the cortex, approximately parallel to the surface of the stem, for varying distances until at their lower end each anastomoses with one of the larger bundles which enter the stele. Thus a great many of these smaller bundles follow a course limited to the cortical region and never directly enter the stelar portion of the stem. Other writers (*13, 30, 40*) have described this cortical vascular system as it occurs in Bromeliaceae (including the pineapple). The lateral leaf-trace bundles are not, however, limited to the cortical region in the pineapple, as BERGMAN and WELLER (13) have indicated.

A conspicuous feature of the stem is the presence of adventitious roots within the cortical region. These roots appear to originate at the boundary between stele and cortex and follow an oblique downward course for some distance through the cortex toward the stem periphery. A radial section of the stem (figs. 9, 10) exhibits very clearly the place of origin of the roots and their course. It appears that there is no definite arrangement of roots, since their proximal ends are found at irregular intervals along the entire boundary between cortex and stele; this may be seen to best advantage by studying retted stems (fig. 11*A*), thick serial sections, both radial and transverse, and by cutting away the cortical region (figs. 9, 12). It seems rather extraordinary to find root primordia, visible to the unaided eye, within 1 cm. (vertical distance) of the apical growing region, and the proximal ends of roots which have already passed through the cortex and are visible at the surface of the stem within 3 cm. (vertical distance) of the apex of the stem (fig. 10*A*). The roots which originated in what is now the lower half of the stem all emerge from the surface, usually in the internodes, to form the soil and axillary root systems. A great number of the latter roots are wound around the stem between the bases of the leaves and the stem. A striking characteristic of the younger, i.e., later-formed, adventitious roots, still within the stem, is that they are apparently already suberized, since various of their elements are colored a reddish brown. This is true even of the roots growing within a few centimeters of the stem apex.

A clearer picture of the gross nature of the more mature portions of the boundary between cortex and stele and of the root "insertion" at this boundary is obtained in a retted stem. An examination of a portion of such a retted stem (fig. 11) shows that the "cylinder" separating the cortex and the stele—which appeared as a translucent or suberized ring in transverse sections and as similar lines in radial sections in the more mature portions of an untreated stem—is made up of a network of anastomosing vascular tissues confluent with the vascular strands which issue from the individual roots. Isolation of a single root shows the numerous vascular strands (the lignified elements only remain after the retting process) which spread out radially after emerging from the root proper (fig. 12*B*). The vascular elements from one root appear to anastomose with those of another and with the vascular network, leaving spaces at intervals (between the areas of the vascular elements) to form the leaf gaps (figs. 11*A*, 12). The bundles from the leaves pass from the cortex into the stele through these gaps; at their

lower ends the elements of these leaf-trace bundles appear to anastomose with the vascular elements of the dividing network and the roots at a lower level.

Radial sections of the stem show what appear to be "pockets" or "islands" of tissue in the nodal regions a millimeter or less beneath the surface (figs. 9, 10, 12). In the apical portion of the stem these so-called "pockets" or "islands" have, in general, been considered to be meristematic tissue from which the axillary buds "originate," being located, as it were, in the "axil" of the leaf where the buds form (73). In the case of more mature portions of the stem they were described as vascular bundles belonging to the lateral buds (13).

In radial sections these areas are either circular or, more often, elliptical in outline, with clearly defined margins (figs. 9, 10, 12). They appear in sharp contrast to the surrounding farinaceous ground tissue because of their translucent appearance. In reality such an area is not actually an "island"—a small isolated group of differentiated cells—but represents a cross section of a circumferential band of what appears to be vascular tissue located just below the surface of the

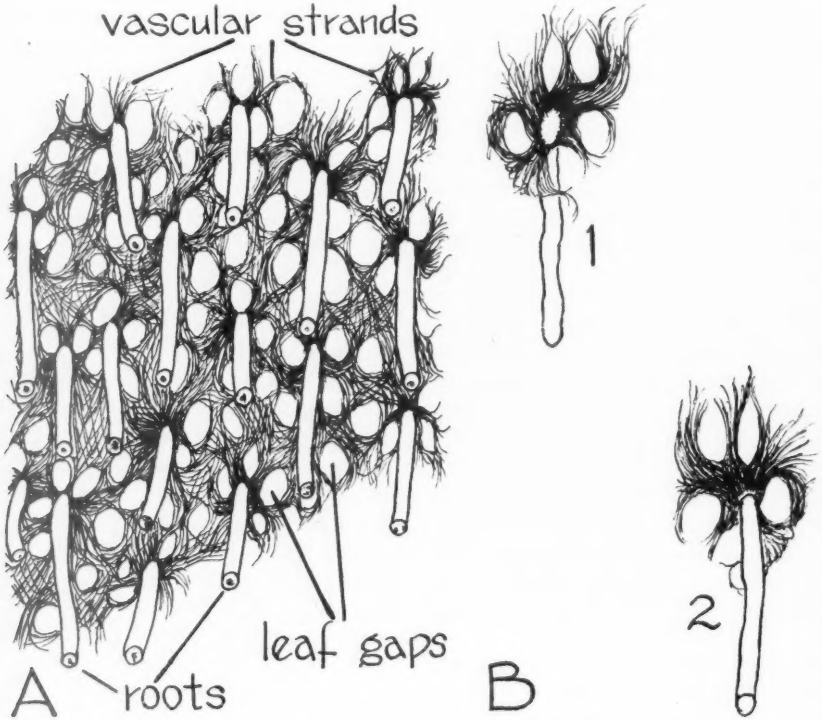


FIG. 11.—*A*, portion of retted stem from 1-year-old plant, showing lignified vascular elements found at boundary between cortex and stele, composed of vascular network and elements issuing from bases of roots. Face view from cortical side. *B*, individual root isolated from *A* to show lignified elements emitting from root base: *1*, view from stelar, *2*, from cortical, side. Approximately ×2.

stem and partly just above and opposite the place of leaf "attachment." The lateral limits of each band appear to be determined by the lateral limits of the insertion of each individual leaf, i.e., the band of vascular tissue extends the same distance around the circumference of the stem as does the base of the leaf inserted immediately below it. The bandlike nature of this vascular tissue can be observed in transverse sections made immediately above the insertion of a leaf (fig. 12) or in a section cut parallel to the surface in the same part of the stem. Such sections represent, of course, longitudinal and tangential views, respectively. The axillary bud is located over this band in a median position with reference to leaf attachment.

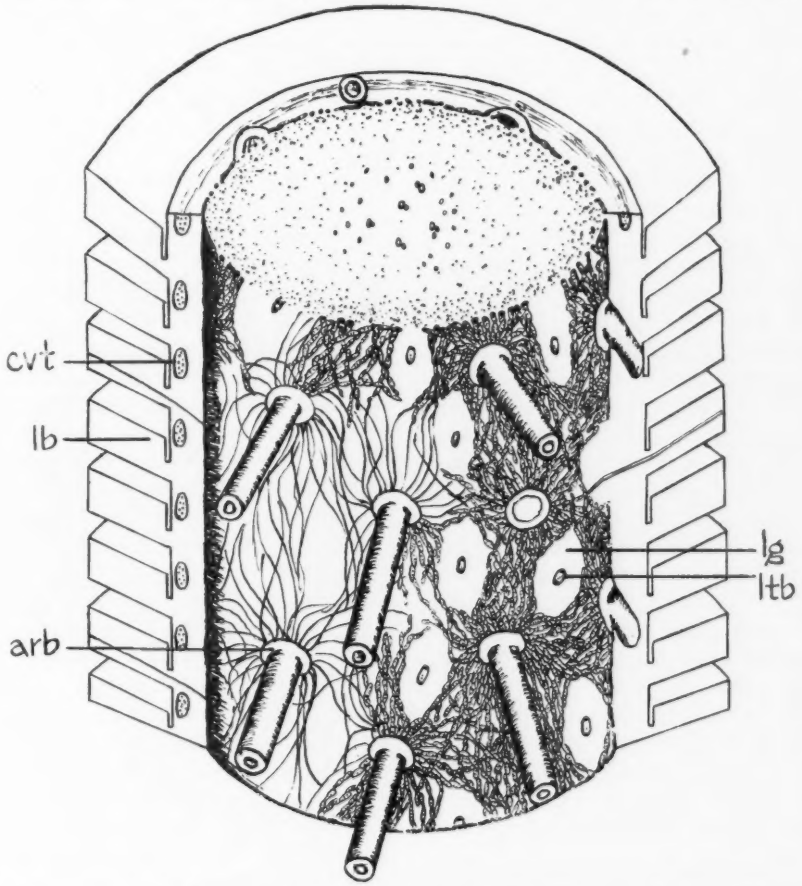


FIG. 12.—Diagrammatic representation of central region of stem of 14-month-old plant, showing portion of cortex cut away to surface of stele to expose vascular network at boundary between cortex and stele. Xylem elements from roots shown. *arb*, adventitious root base; *cvt*, circumferential band of vascular tissue; *lb*, leaf base; *lg*, leaf gap; *ltb*, leaf trace bundle. Slightly larger than natural size.

APICAL MERISTEM, OR GROWING REGION

The following interpretation of the apical meristem of the pineapple stem[10] is based partially upon the work of previous authors who have described bromeliaceous and other monocotyledonous stems of a similar type (**9, 13, 16, 91, 101, 103, 115, 123**) but primarily on personal examination. It is hoped that this description of the apical meristem in the pineapple stem may facilitate an interpretation of that in many other monocotyledonous stems.

In this description the term "apical meristem" will be used in the broadest and, to us, the most logical meaning of the phrase to include all parts of the apical growing region: (1) the terminal meristem, consisting of (*a*) the acroscopical portion which is undifferentiated apical tissue; this merges gradually basiscopically into (*b*) the older differentiating but unspecialized tissue,[11] which in turn passes into (2) the specialized meristematic tissue, the dictyogenous layer, which lies between the cortex and stele. All these represent successive stages of a primary meristem which builds primary tissue.

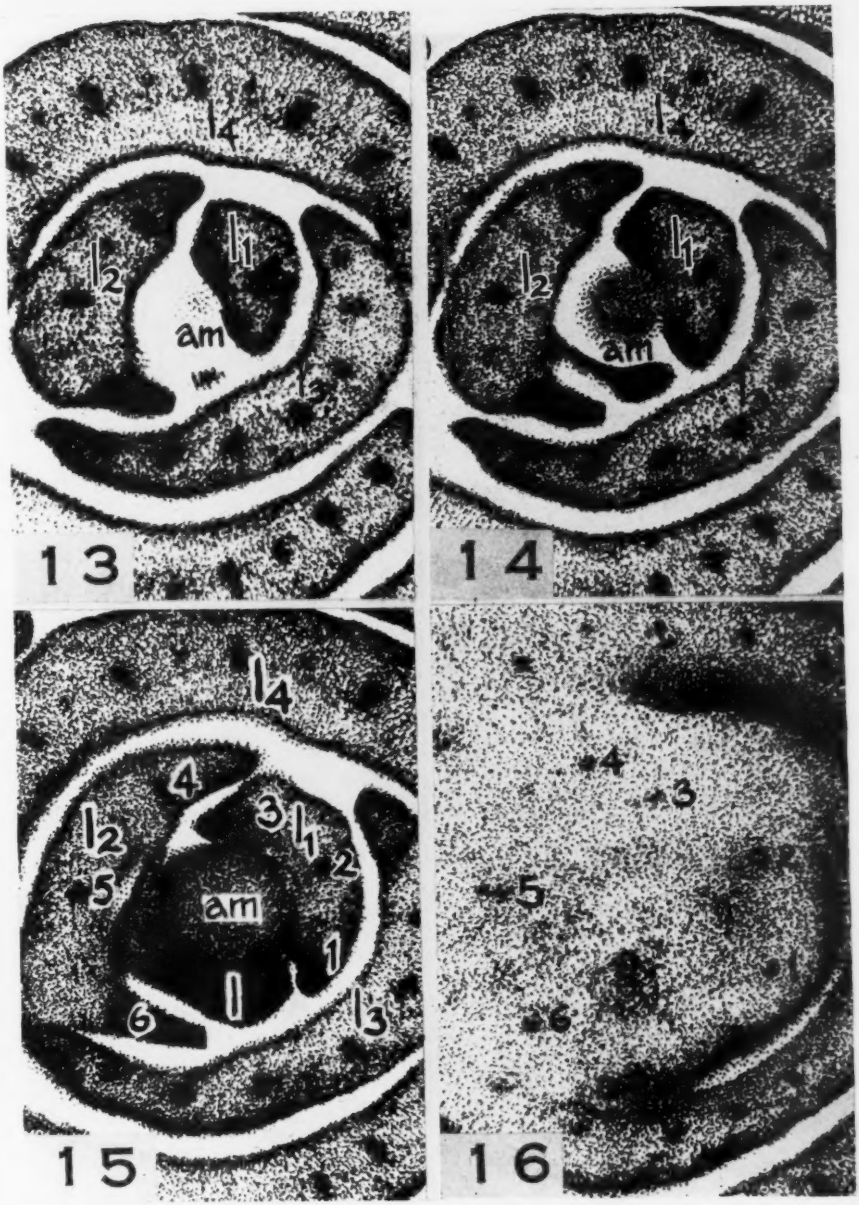
The acroscopical portion of the terminal meristem is in the form of a spherical segment above the latest-formed leaf primordium (figs. 13, 14, 15) and in radial section exhibits a relatively broad domelike, slightly convex appearance (figs. 22, 23*A*, 32). As in most plants, the cells in this portion, essentially alike, are approximately isodiametric in form and without intercellular spaces. The walls are thin and of cellulose; the protoplasm is abundant; the nuclei are prominent; and vacuoles are lacking (fig. 23). The initiating cells are probably subterminal and apparently indefinite in number. The outer row of cells, the dermatogen, forms a definite and discrete layer over this portion (fig. 23). Functionally, this portion of the terminal meristem is the zone of cell initiation.

In the transition from the youngest meristematic cells to differentiating meristem, a change which is gradual and without sharp demarcation, a lateral expansion or projection which is a leaf primordium and, soon after, a procambial strand are formed (figs. 15–20, 22, 24). The cells in the latter are distinguished from the remaining, undifferentiated meristematic cells chiefly by their narrow and elongate shape; they also usually have denser protoplasm (figs. 20, 22, 24). This procambial strand becomes the first leaf-trace bundle for the latest-formed leaf primordium; it continues into the leaf as the central procambial strand there (figs. 22, 24). Procambial strands for leaf-trace bundles in a position immediately lateral to this bundle are formed with progressive development of the meristem.

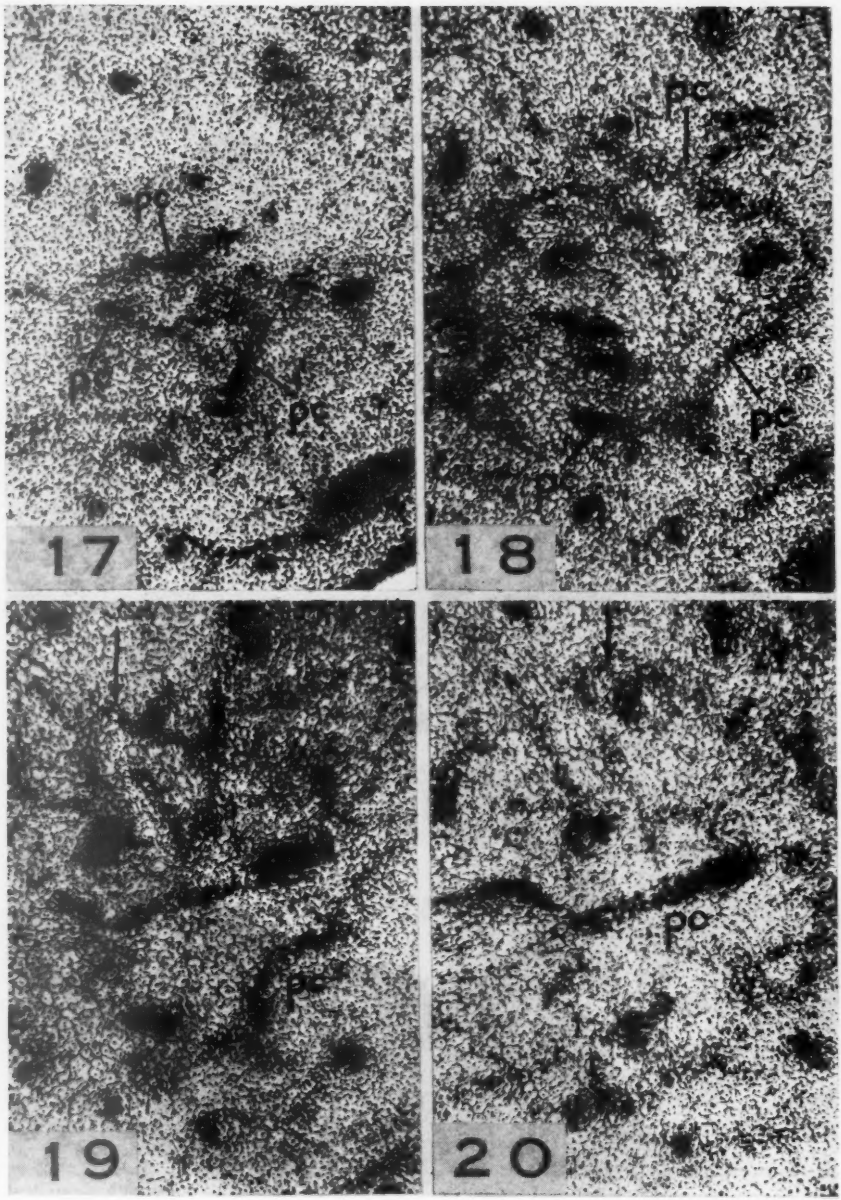
Differentiation in the terminal meristem begins in the center of the stem and progresses centrifugally, so that at progressively greater distances back from the apex a core of differentiated parenchyma cells and vascular bundles, constantly increasing in diameter, remains as the zone of diminishing meristem becomes more and more restricted toward the periphery of what will be the central

[10] It should be kept in mind that the stem of a plant just before floral differentiation—about 14 or 15 months after planting of the propagating material—is being described here so that this is a vegetative meristem, i.e., one in which foliar histogenesis only is proceeding.

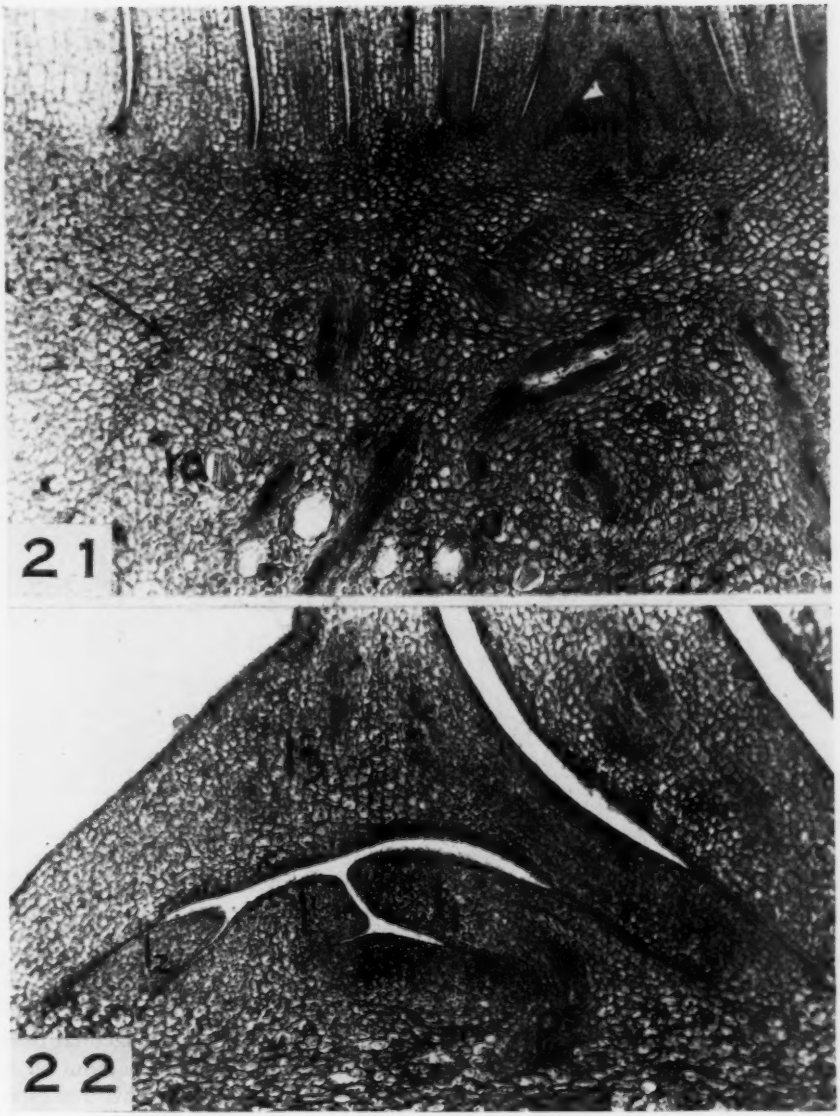
[11] A distinction between promeristem and meristem proper has been made by various authors. In the pineapple stem the acroscopical portion of the terminal meristem, above the latest-formed leaf primordium, or including the latter in the opinion of some authors, could be designated as promeristem and the criteria of many authors satisfied; actually, there are no definite limits within this terminal meristem.



FIGS. 13–16.—Apical meristem. Series of transverse sections of stem apex of 7-month-old plant. Fig. 13, cut at tip of acroscopic portion, and figs. 14–16, cut at successively lower levels back from tip (each 20 μ below preceding section). *am*, acroscopic portion of apical meristem; *l*, last-formed leaf primordium; l_1, l_2, l_3, and l_4, successively older leaf primordia. Numbers indicate same procambial strands of second and third youngest leaf primordia in figs. 15, 16.



FIGS. 17–20.—Differentiating apical meristem. Series of transverse sections of stem apex of 7-month-old plant, each section cut 20 μ below preceding section, to show differentiating procambial strands, *pc*. Section shown in fig. 17 cut 20 μ below that shown in fig. 16.



FIGS. 21–22.—Apical meristem. Fig. 21, radial section of apical region of 13-month-old seedling, and fig. 22, of 4-month-old plant. *am*, acroscopical portion of apical meristem; *l*, latest-formed leaf primordium; l_1, l_2, l_3, successively older leaf primordia; *ps*, procambial strand differentiating in apical meristem and next to youngest leaf primordium; *ra*, raphides cell. Arrow points to meristematic zone between cortex and stele in which later-formed procambial strands are initiated.

cylinder or stele. Since development within the region which becomes the cortex is to some extent centripetal, a zone of meristematic tissue is left between the cortical and stelar regions (figs. 21, 26). In this portion of the terminal meristem, then, procambial strands with interlying parenchyma are formed, first in the central portion of the stem (figs. 17–20) and then, with further development of the stem—at different levels back from the apex—in a narrow zone at progressively greater distances from the center (figs. 21, 26, 29*A*, 32). In this narrow zone of meristematic tissue additional procambial strands and parenchyma develop toward the inside (fig. 30).

It is often difficult at first to recognize the cells of this narrow meristematic zone from the adjacent stelar and cortical cells (figs. 29*B*, 30). The zone is perhaps most easily located in radial sections viewed under lenses of low magnification (fig. 29*A*) or in seedling stems (fig. 21). In both radial and transverse sections the cells appear as a narrow zone slightly denser than the surrounding tissue (figs. 21, 26, 29); they can sometimes be seen to somewhat better advantage in oblique sections because they form a wider zone in such a section. Examination under high magnifications shows that the cells of this zone are primarily characterized by being narrower in a tangential plane than adjacent cells and without intercellular spaces (fig. 31); this makes for the denser appearance of the zone under low magnification.

It should be emphasized that the terminal meristem just described actually extends over a very short vertical distance in a stem of the age under consideration.

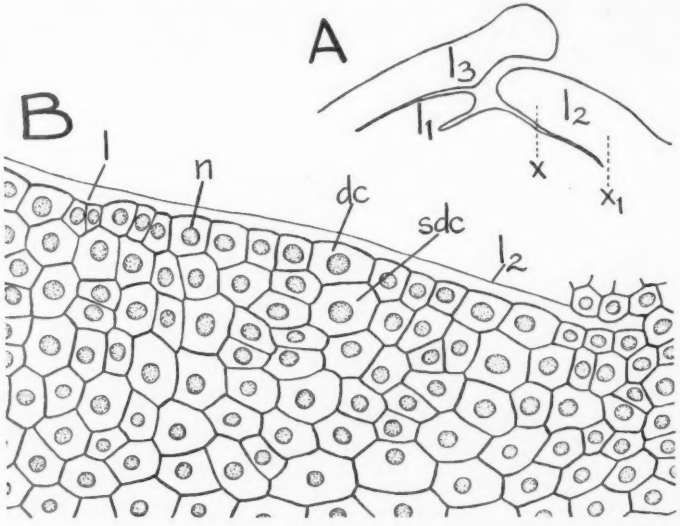
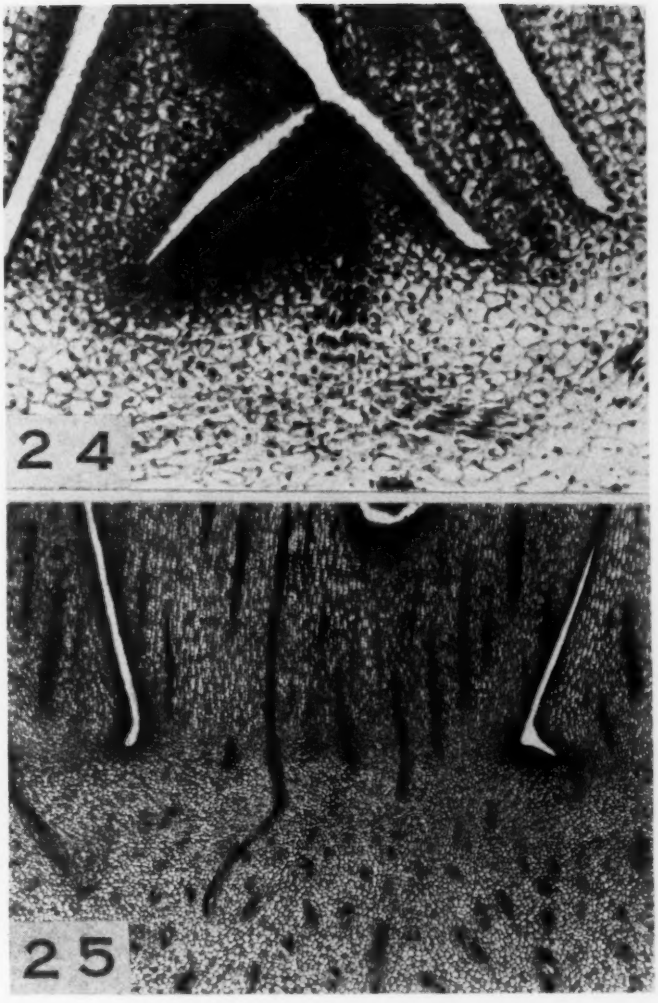
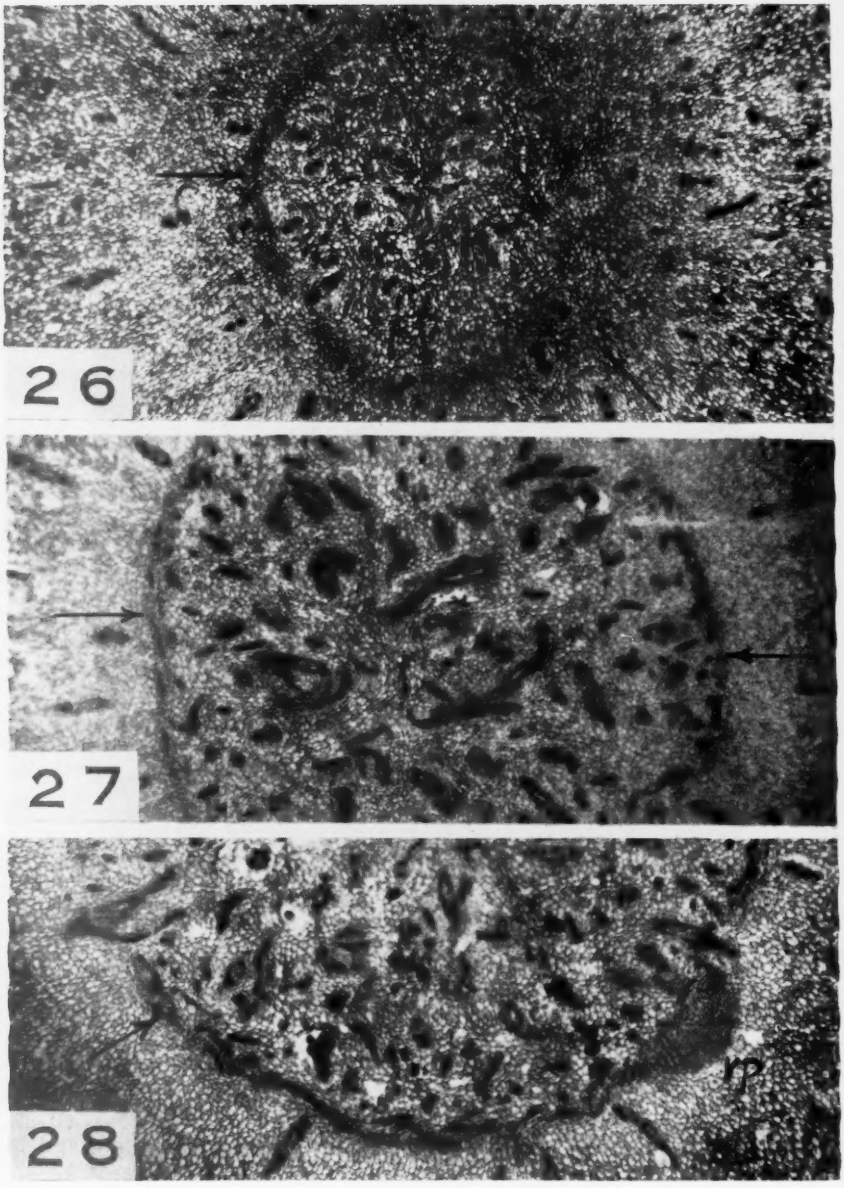


FIG. 23.—View of earliest stage of leaf initiation visible, in radial section of 4-month-old plant. Zone delimited by broken lines X, X_1 in A and shown enlarged in B is that in which leaf is being initiated. *dc*, dermatogen cell; *sdc*, subdermatogen cell; *n*, nucleus; *l*, cell division in dermatogen, indicating leaf initiation; l_1, l_2, l_3, successively older leaf primordia.



FIGS. 24–25.—Longitudinal sections of stem apex to show, fig. 24, tangential view of leaf primordium and differentiating procambial strand—this will become median leaf trace—and, fig. 25, to show tangential view of older leaf with other differentiating strands, lateral to median trace.



FIGS. 26–28.—Meristematic "ring" in stem. Series of transverse sections of 13-month-old seedling stem at successively lower levels back from apex to show ring of meristematic tissue (*indicated by arrows*) at periphery of stele in which, fig. 26, procambial strands and parenchyma are formed (these procambial strands become leaf trace bundles), and, figs. 27, 28, specialized meristem—dictyogenous layer—in which vascular network between cortex and stele, and adventitious roots (*rp*) are initiated. Arrows point to meristematic tissue. *Section in fig. 26 cut 0.3 mm. below tip of stem*; in fig. 26, 0.7 mm., and in fig. 28, 1.0 mm. below tip of stem.

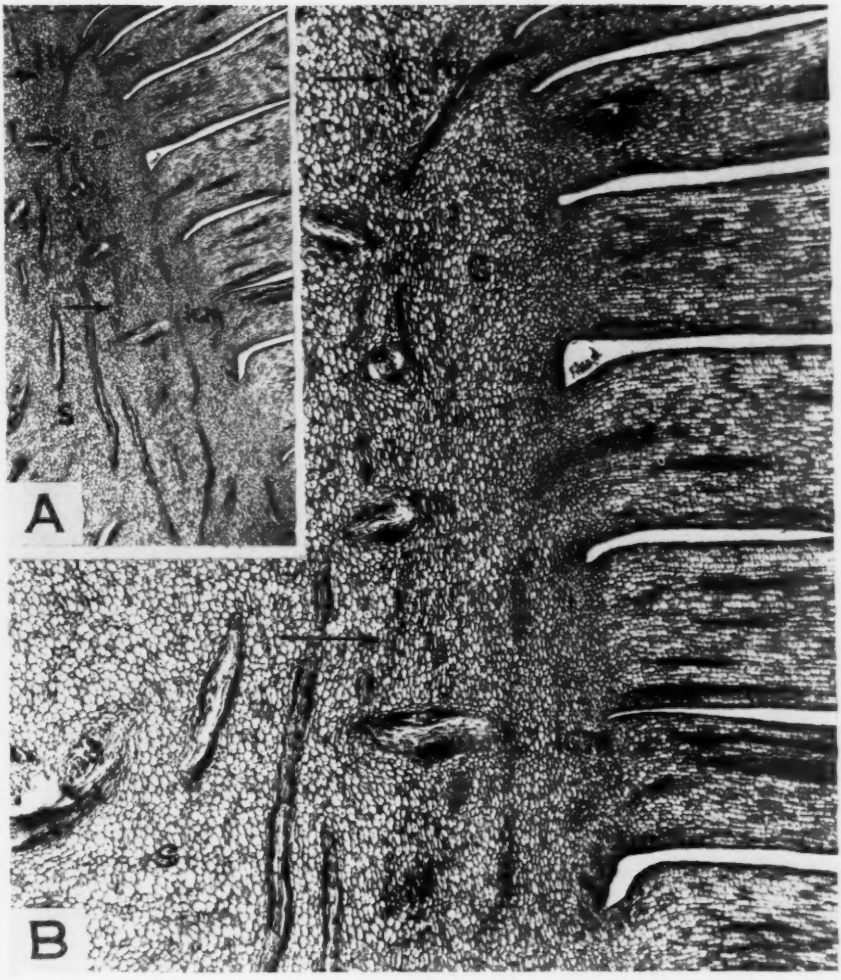


FIG. 29.—Longitudinal section of stem of 7-month-old plant cut near apex to show meristematic tissue lying between cortex and stele. Arrows point to procambial strands forming in meristem. *A*, low, and *B*, high, magnification. *icm*, "intercalary" meristem at base of leaf; *ltb*, leaf trace bundle entering leaf; *c*, cortical and *s*, stelar, tissue.

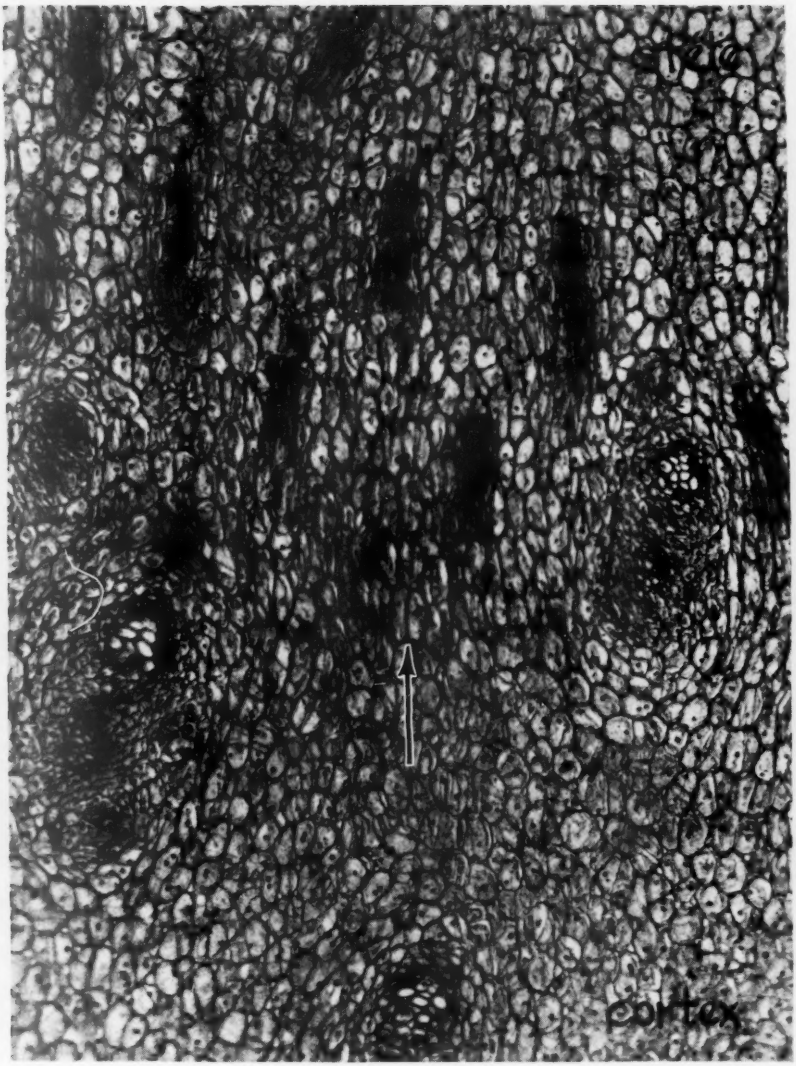


FIG. 30.—Procambial strands initiating in meristematic tissue between cortex and stele. Meristematic zone "moves away" from initiated strands as procambial strands and interlying parenchyma become differentiated. Arrow indicates meristem.

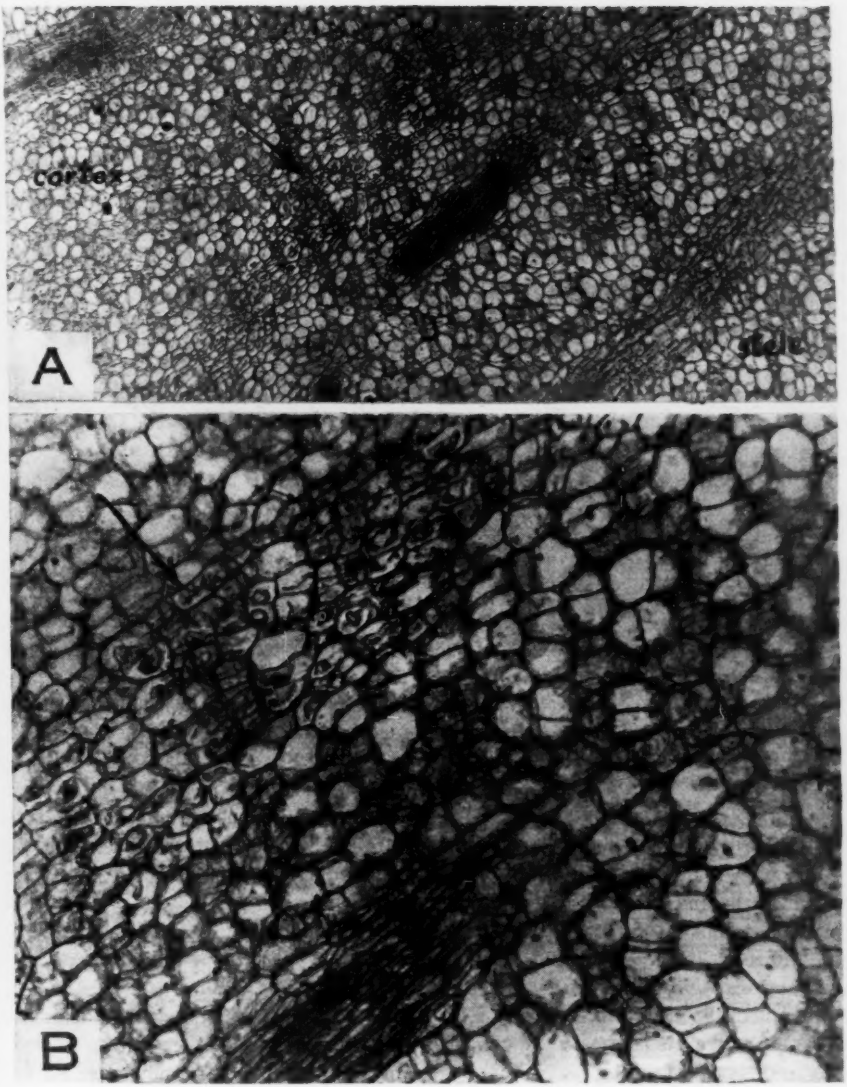


FIG. 31.—Apical meristem. Longitudinal section of stem to show meristematic tissue (indicated by arrow) at boundary between cortex and stele, and procambial strand recently initiated in it. *A* low, *B*, high, magnification.

When the function of differentiating procambial strands which become the leaf-trace bundles and the interlying parenchyma is completed, this meristematic sheath passes over into a meristem with a specialized function. This basiscopic portion of the apical meristem, in the form of a narrow sheath between cortex and stele (figs. 27, 28), is a tissue

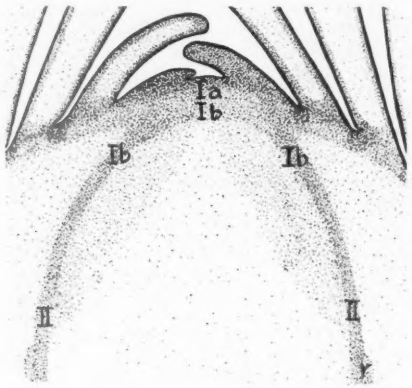


FIG. 32.—Diagrammatic drawing to show different parts of apical meristem: *I*, terminal meristem consisting of *a*, acroscopical portion—undifferentiated apical tissue—and *b*, differentiating but unspecialized meristem. *Ib* passes over into *II*, specialized meristematic tissue lying between cortex and stele—MANGIN's dictyogenous layer. With final activity of latter, root primordia (*r*) are initiated. Vertical distance greatly exaggerated.

similar to the dictyogenous layer in some monocotyledons described by MANGIN (**91**). JACKSON (**67**) included this term in his botanical glossary, describing this layer, not quite fully and correctly, as a meristem which gives rise to the central body and cortex of young roots in monocotyledons; the term "dictyogenous" itself is usually applied to monocotyledons with netted veins (from the Greek *diktyon*, "net"). The function of this specialized meristem, the dictyogenous layer, is twofold: in it (*a*) are differentiated the procambial strands which develop into the vascular network which serves as the intermediary between the leaf-trace bundles and the root-vascular system, and (*b*) the adventitious roots arise.

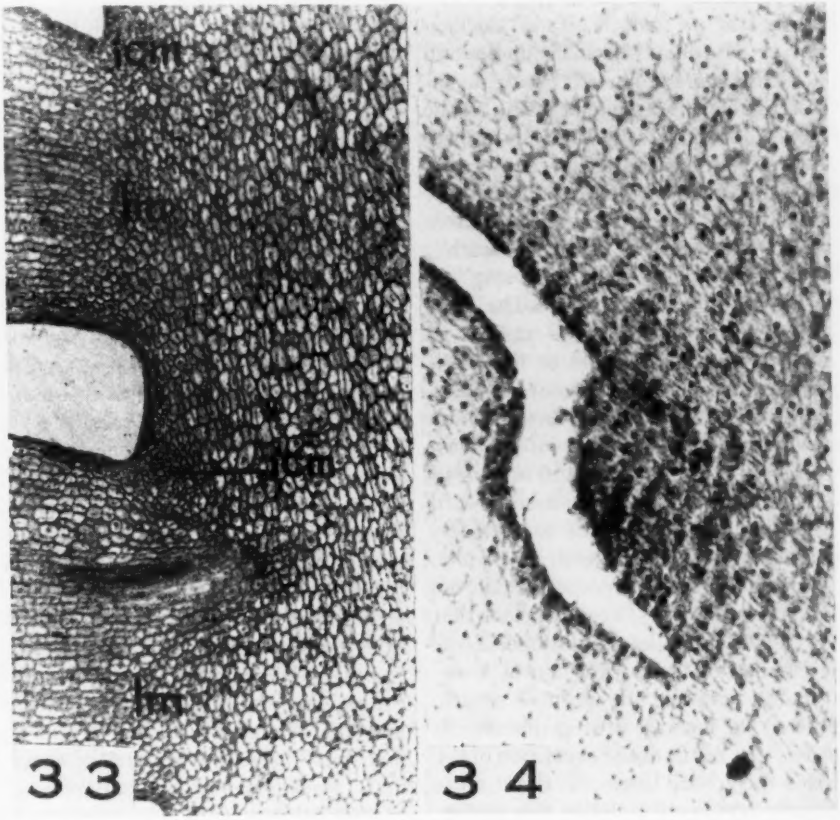
The apical meristem of the pineapple stem as a whole may be represented by a figure consisting of a dome, solid at the summit but becoming "hollowed" to form a broad-based "cavity" in following the slopes of the dome in a basiscopic direction, with the base finally extended to form a hollow cylinder. The terminal meristem corresponds to the dome-shaped part of the figure, the acroscopic portion forming the summit, and the differentiating meristem the sides of the dome. The specialized meristem—the dictyogenous layer—forms the side of the hollow cylinder (fig. 32). This "figure" actually covers not more than 2 cm. in vertical distance in a stem of the age under consideration.

As the terminal meristem advances with the elongation of the stem, a portion of the tissue in the axil of each leaf remains meristematic (fig. 33). These meristematic regions represent the so-called "intercalary meristems" of some authors. The lateral-branch-bud initials arise from this meristematic tissue (fig. 34). The basal portion of a leaf and the confluent stem tissue remain a meristematic region for some time, the former until the leaf is fully developed; these regions may also be said to represent an intercalary meristem.

The type of apical meristem described as occurring in the pineapple stem probably occurs in all Bromeliaceae. Except for some inconsistencies, BERGMAN and WELLER's (**13**) fundamental conceptions and interpretation of the apical meristem in the pineapple stem were correct as far as they went. PETERSEN (**103**) recognized a meristematic zone between cortex and stele, and BORESCH (**16**) gave a fairly ac-

curate description of the meristem and its functions in other Bromeliaceae, but both authors unfortunately used the terms "primary meristem" and "cambium" for this meristem without any discrimination between the two. The confusion created by their use of these two terms to describe this apical meristem later led SOLEREDER and MEYER (141) to refer to these authors' descriptions as of "a secondary growth in thickness" in bromeliaceous stems.

In the literature on other monocotyledonous plants the apical meristem described is probably in many cases similar to that found in the pineapple and other Bromeliaceae, but the unfortunate use of terms by many of the authors of such description makes this obscure. Early investigators of the apical meristem of monocotyledonous stems were at first undoubtedly influenced by contemporary conceptions regarding this tissue in dicotyledonous stems. Thus SCHLEIDEN



FIGS. 33–34.—Fig. 33, longitudinal section of stem of crown (leafy terminus of main axis beyond fruit) to show meristematic region ("intercalary" meristem, *icm*) remaining from apical meristem in axil of leaf; in this region lateral branch bud (fig. 34) is initiated. *lm*, "intercalary" meristem (meristematic region) at base of leaf.

(121), the first to study this type of monocotyledonous stem (one with primary growth only), and others who followed (51, 71, 101, 115, 116, 123, 145), described monocotyledonous stems in general, or specific ones, as having a "cambial sheath" or "mantle," or a "thickening ring," in which vascular bundles and parenchyma are produced toward the inside, thus contributing temporarily to the increase in thickness of the stem. Lower in the stem this meristem, in many cases, was said to develop into a permanent tissue which some of the investigators called the "lignified cambial sheath"; others, a "lignified thickening ring" or simply a "lignified cylinder." The error made by these investigators was not so much in their description of the meristematic tissue, for in almost all cases their observations were correct as far as they went, but in their use of the term "cambium" for this tissue and in some of their interpretations. They used a word ordinarily associated with a *secondary* meristem to describe a *primary* meristem.

In contrast to the conceptions referred to above, others (35, 102) believed that the vascular bundles are formed in the meristem constituting the apical growing-point, without reference to a sheath, and that the increase in stem diameter is entirely the result of the expansion of the cells in the ground tissue and those of the vascular bundles formed in this region. In a way the observations of these authors are correct as far as they went, but still they did not have the complete picture. They did make a valuable contribution, however, in condemning the use of the terms "cambium" and "cambium sheath" in connection with this type of monocotyledonous stem.

FALKENBERG (40) and PETERSEN (103) believed that both the above types of growth in thickness—with and without a definite sheath—may occur in different representatives of the group of monocotyledons with a primary meristem. This led BORESCH (16) to divide all monocotyledons "with a definite or limited primary increase in diameter" into these two groups. He used the word "cambium," but he was definitely writing of a primary meristem.

Others, like DEBARY (30), adopted HANSTEIN's terminology of "dermatogen," "plerome," and "periblem," which is entirely unsuited for the monocotyledonous stem.

Unfortunately, many of the more recent authors have added little to clear up the confusion. They have, for the most part, been content to adopt the terminology and ideas of the earlier investigators, with little additional original research to fill in the details on the nature of the apical growing regions of monocotyledons with primary meristems. ARBER (2) wrote of this meristem as a "procambial mantle"; SKUTCH (139) "doubted the propriety of calling this meristem a cambium" but decided to retain it in his description of the banana stem "for want of a better word." BALL (9) accepted SANIO's "thickening ring" and SKUTCH's "cambium" but used his own designation "cambium-like primary thickening meristem" in his descriptions of the primary thickening growth and origin of "provascular strands" in palm stems. ESAU's (39) use of the term "primary thickening meristem" for this tissue in a review of the primary growth of monocotyledons is commendable; she wrote, however, of a "cambium-like lateral meristem" and an "annular meristematic zone" as two different types of meristematic tissue without making clear what these differences are. SIFTON (137) contented himself with a review of the literature on what he called the "residual meri-

stem" (adopting KAPLAN's [70] term) and the "procambium"; he did not discuss the dicotyledonous and monocotyledonous stems separately.

MANGIN (91), although not always consistent in his terminology and with several inaccuracies in his paper, has probably given us the most nearly correct interpretation of the primary meristem in the axes of stems of the type under consideration. Many of the other authors had basically the right idea but through the adoption of inappropriate or incorrect terminology, and by not following their studies through far enough, have not given us so clear a picture as did MANGIN. He used the term "primary meristem" to cover both the tissue usually designated as promeristem and the sheath of meristem basiscopic to this. The authors (71, 101, 115, 116, 121) who first described the so-called "cambium sheath" or "thickening ring" may well have had a primary meristem in mind, as some of their descriptions indicate, but because they unfortunately used a term, "cambium," previously used only in connection with descriptions of stems with secondary growth, other authors (35, 40, 102, 103, etc.) who followed them assumed that they had a secondary meristem in mind. MANGIN described the change from meristematic (promeristematic) to permanent tissue as beginning simultaneously in two regions: in the center and at the periphery of the stem. In the central region differentiation proceeds in a centrifugal; at the periphery, in a centripetal direction. As a result, the primary meristem becomes gradually reduced to a ring of meristematic tissue separating a central cylinder and a cortex. This meristematic ring passes over into a generative layer which he called the "dictyogenous layer" (*couche dictyogène*); this layer is responsible for the formation of the "radiciferous network" (*réseau radicifère*)—the vascular network lying between cortex and stele—and adventitious roots.

Before leaving the subject of the apical meristem, some reference should be made to the tunica-corpus theory. In recent years the earlier, long-accepted terminology of HANSTEIN, who divided the meristem into three different histogens (the dermatogen, which forms the epidermis; the plerome, which produces the central cylinder; and the periblem, a region lying between the first two, which usually forms the cortex and sometimes the pericycle and vascular tissue as well), has been replaced to a great extent by that of SCHMIDT (122), who was concerned primarily with the genesis and growth of cells in the meristematic region itself. SCHMIDT proposed the term "tunica" to designate the layer consisting of the apical external row or rows of cells which ordinarily divide in the anticlinal plane except during leaf or bud initiation and the term "corpus" to include all the remaining internal tissue in which divisions commonly occur in diverse planes. Thus HANSTEIN's "dermatogen" and "periblem" are united under the collective term "tunica." Many recent authors (3, 8, 27, 28, 43, 44, 45, 60, among others) have accepted SCHMIDT's theory because "the concept of the tunica is sufficiently plastic to include those types of shoot apices in which only a single discrete surface layer is maintained" and because "the tunica-corpus theory serves to focus our attention upon the dynamic rather than the purely formal aspects of the shoot apex, in that the demarcation of zones reflects the distribution of well-coordinated growth patterns which may be expected to fluctuate within wide limits in the various groups of the angiosperms" (45).

Whether one or more layers of cells in the acroscopic portion of the terminal meristem of the pineapple stem can be selected to form a distinct tunica is open to question in the opinion of the present writer. The outermost row of cells forms a distinct layer, it is true, but since the term "dermatogen" has for a long time been used to designate this primordial epidermal layer and is appropriate, it hardly seems necessary to adopt a new term. If the term "tunica" is not adopted, there is no point in retaining that of "corpus." Since HANSTEIN's terms are also not applicable to the pineapple stem, or to monocotyledonous stems in general, the simple terminology of *dermatogen* and *subdermatogen* cells has been used in this paper.

THE VASCULAR CYLINDER SEPARATING CORTEX AND STELE

The network of vascular tissue which separates the cortex and stele is formed in the specialized meristem, the dictyogenous layer; the initiation in this meristem of the procambial strands which develop into this network represents the final activity of the apical meristem (fig. 28). The procambial strands differentiate and develop entirely within the narrow limits of this meristematic tissue. Their course is directed variously—vertically, horizontally, and obliquely—but within the plane coincident with that of the stele-cortex boundary.

The strands of vascular tissue consist chiefly of xylem with very little phloem. Associated with the bundles, and also formed in the dictyogenous layer, are parenchyma cells different from adjacent or near-by stelar and cortical parenchyma. Those parenchyma cells lying between the individual strands of vascular tissue are large and irregular in shape, with the longer axis in one of several directions. The longer axis is often not much greater than the other. The parenchyma cells lying just internal to the vascular network are narrow cells, elongated in a direction parallel to the stem axis; these cells are narrower and longer than adjacent stelar parenchyma (fig. 35).

Outside the vascular network and actually forming the peripheral limits of the stele are several rows, numbering from two to four, of cells which deserve special attention. They also are formed in the dictyogenous layer. In the early stages of development they are seen as very narrow, elongated cells, conspicuous in contrast to adjacent cortical parenchyma (fig. 35). The walls of the outermost of these cells eventually become thicker, resembling the sclerenchyma cells which form the large caps on the leaf-trace bundles, although the walls are not quite so thick as in the cap sclerenchyma. The walls become lignified and, in the older portions of the stem, suberized. The walls are profusely pitted (figs. 36, 37), the pits being simple and oriented obliquely (figs. 36, 39). The walls of the cells in the several other rows lying between this row of sclerenchyma cells and the strands forming the vascular network are of a similar shape as those in the row just described, but the walls do not become lignified nor are they pitted.

These rows of cells just external to the vascular cylinder have been variously interpreted in the Bromeliaceae. Most authors (**13, 16, 40, 91, 93 141**) have considered the outermost of these rows an endodermis, with the cells lying inside it, the pericycle. JÖRGENSEN (**69**) and MEZ (**94**) described it simply as a layer of well-developed or greatly thickened sclerenchyma. It is perhaps not too surprising that so many authors described the presence of an endodermis and pericycle:

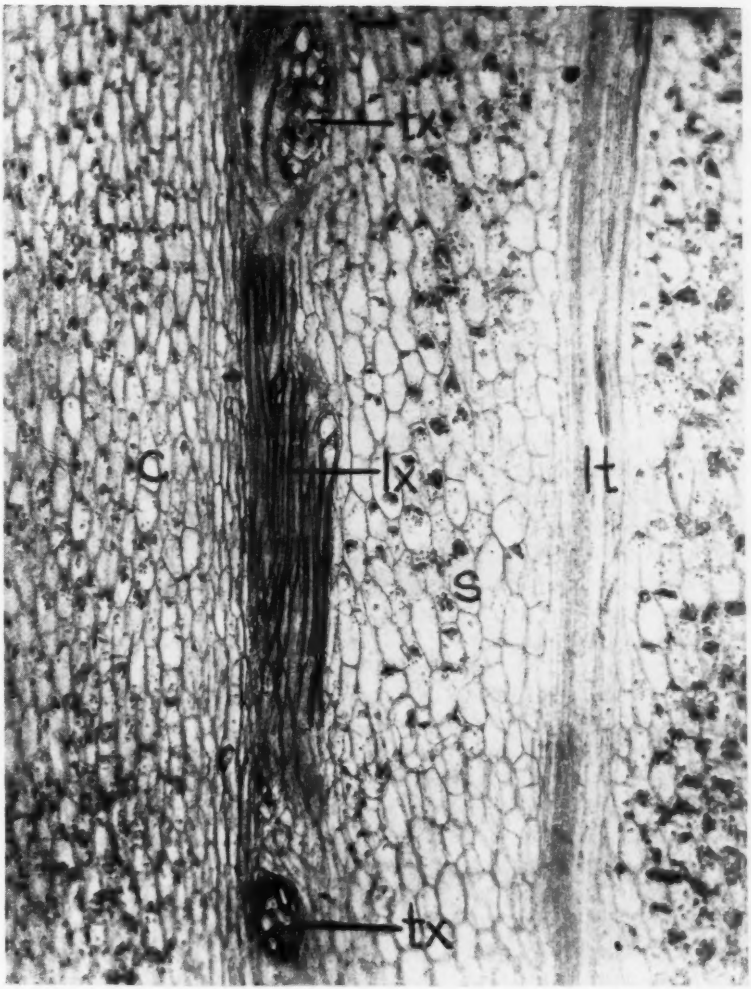
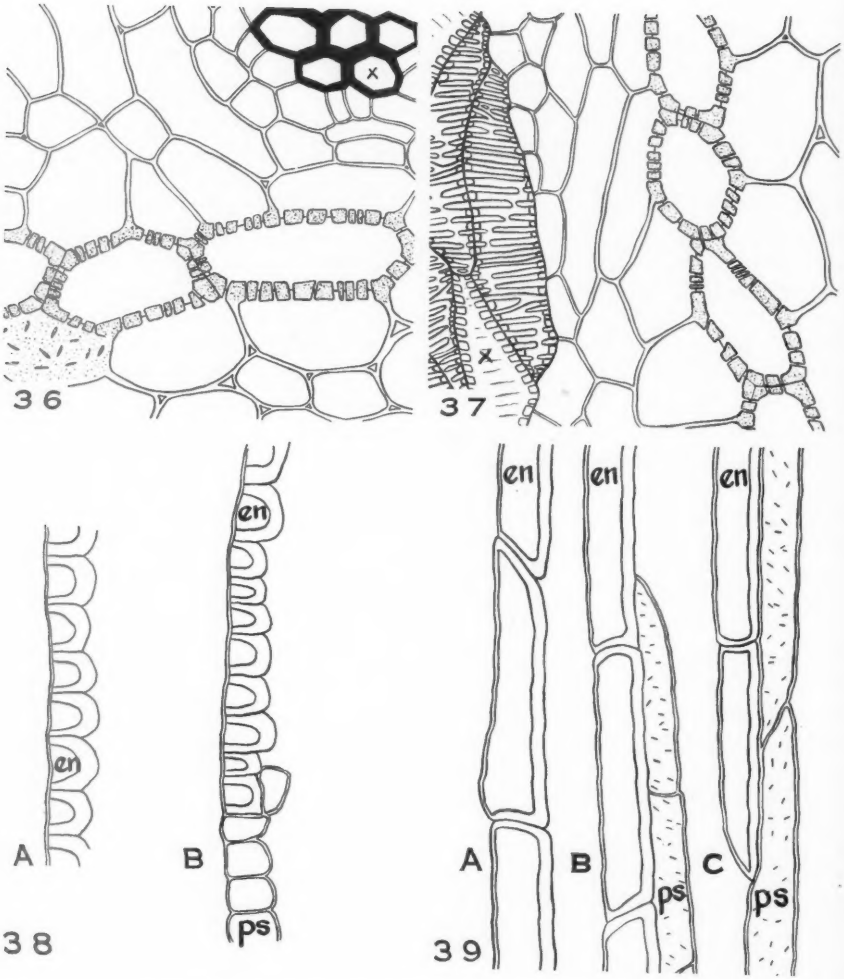


FIG. 35.—Longitudinal section of stem showing elements of vascular network at boundary between cortex and stele: *lx*, xylem cut in longitudinal, and *tx*, in transverse, section; *c*, cortex; *s*, stele; *lt*, leaf trace. Black specks in parenchyma cells are starch grains. Limiting the vascular network tissue on cortical side are long narrow cells which become peripheral sclerenchyma.



FIGS. 36–39.—Peripheral sclerenchyma cells of stele. Fig. 36, transverse and, fig. 37, longitudinal sections of mature part of stem showing sclerenchyma cells at periphery of stele (cells with stippled walls)—usually erroneously called "endodermis." These cells are located just externally to elements of vascular network (*x*, xylem) at boundary between cortex and stele. Figs. 38, 39, transverse and longitudinal sections, respectively, of cells of endodermis (*en*) of root (*A* in figs. 38, 39), and peripheral sclerenchyma cells (*ps*) of stele and transitional type of cells (*B* in fig. 38 and *B* and *C* in fig. 39).

first, because it has almost become a classicism even in descriptions of monocotyledons to consider that, if a stem is divided into cortex and stele, there must be present a pericycle and endodermis limiting the stele at its periphery; and, second, because in superficial examination of radial sections of stem tissue containing adventitious roots, the row of endodermal cells in the root appears to be continuous with a row of cells which could be the stem endodermis. Careful examination will show, however, that the cells of the so-called "endodermis" of the stem are quite different from the endodermal cells of the root and in no way seem to fulfil the qualifications of stem endodermal cells except in shape. They are not arranged in a single continuous row; their walls are not thickened in the characteristic endodermal manner; and they are profusely pitted—all these features represent great divergences from the usual endodermal cells.

By following the endodermis in a longitudinal section of the root back to its basiscopic portion in a stem section, one finds that, in the region where the outer cortex of the root ends, the transition from the true endodermal cells of the root to the "endodermal-like"—the peripheral sclerenchyma—cells of the stem stele is gradual. We find the single row of endodermal cells passing over to a double row, one of endodermal cells and the other of endodermal-like cells, which in turn passes over into a single row of the sclerenchyma cells (figs. 38, 39). At best we could call these cells in the stem "endodermis-like" cells, as have several other authors, because of their position and shape. Thus, these peripheral sclerenchyma cells, as this writer prefers to call them, of the stele of the pineapple stem may, in a way, be considered to serve a function similar to the endodermis in other stems.

It is usual to consider the layer inwardly adjacent to the endodermis as pericycle, potentially meristematic tissue. Some authors have assumed that the roots in bromeliaceous plants, including the pineapple, are initiated in this tissue, as in some other plants. That this is not true will be shown later in the description of the root; and there is, therefore, no functional "need" for a pericycle in the pineapple stem.

The vascular elements of an adventitious root issue from the basiscopic portion, spread out along the outer surface of the vascular network, and anastomose with the elements of the latter. On the inner surface of the network the elements of the lower ends of the leaf-trace bundles anastomose with the elements of the vascular cylinder. Thus, the vascular network, the vascular strands from the roots, and the vascular elements of the leaf-trace bundles become an interjoined system, the vascular network functioning as an intermediary between the other two vascular units.

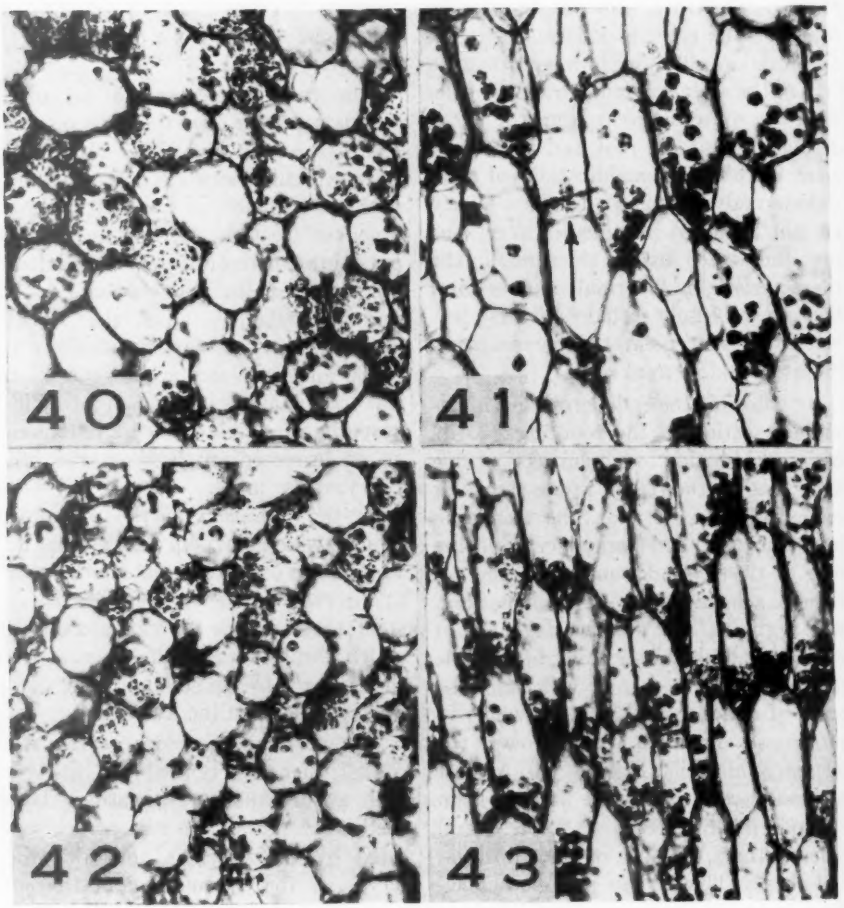
In the initiation of the procambial strands which make up the vascular network, areas of nonvascular tissue are left at intervals in the differentiating meristem; these become the leaf gaps through which the leaf-trace bundles pass from the cortex into the stele on their downward course from the leaf.

The permanent tissues of the stem consist, then, of a central core, the stele, with a surrounding ensheathing layer, the cortex. These two regions are separated by the vascular network which serves as the vascular connection between the roots and leaf-trace bundles at their lower ends. Limiting the stem externally is the epidermis.

THE STELE, OR CENTRAL CYLINDER

The stele consists, essentially, of a great mass of ground tissue and the vascular bundles imbedded within this. Since the vascular system of the stem is described as a whole in a later section, that portion which lies within the stelar region is not described here. There is no indication within the stele of a differentiation of the stem into nodal and internodal regions.

The ground tissue is made up of a rather uniform parenchyma (figs. 40, 41). The cells in any small region examined seem to lie in longitudinal rows but are actually rather irregularly arranged. At first glance these cells appear rather compactly arranged; closer examination,



FIGS. 40–43.—Transverse, fig. 40, and longitudinal, fig. 41, sections of cortical ground tissue; transverse, fig. 42, and longitudinal, fig. 43, sections of stelar ground tissue of stem. Most cells contain starch grains. Arrow in fig. 41 points to truncate end wall of cell which results from nonrounding-off of end walls of daughter cells in division of mother cell.

however, shows that, since the end walls are usually somewhat rounded, intercellular spaces are present. The prevailing shape of the cells in longitudinal sections is ellipsoidal, with the greater diameter parallel to the stem axis (fig. 41). Frequently, in the development of this tissue, when a cross wall forms in the mother cell, both end walls of the daughter cells do not become rounded, so that, while one end is rounded, the other is truncate (fig. 41). In such cases the diameter parallel to the stem axis is definitely less than in the case of the ellipsoidal cells.

The cells of the parenchyma are filled with starch grains and are often tightly packed with them (figs. 40, 44). Both ellipsoidal and spherical—especially the latter—simple grains of starch occur in these cells; more frequently, the grains are compound (fig. 45), and both these and the simple type are often present in the same cell. According to the general classification of compound starch grains (57), those in the pineapple can be further described as being completely compound—the planes of separation between the component grains extend to the surface. The component grains form semiangular, partially rounded segments, having two or more plane surfaces which result from the mutual pressure of the individual grains. The compound grains are often seen broken up into their component parts, within the cells; when this is the case, their semiangular form becomes apparent (fig. 45). According to STRASBURGER (145), such a compound starch grain results from several grains commencing to form in a single leucoplast; this causes their crowding. The hilum in the pineapple starch grain is usually either forked or stellate and is highly refractive to light (fig. 44). The layering in the grains is rarely discernible.

The walls of the stelar parenchyma cells are of cellulose.

Scattered throughout the parenchyma just described are isolated cells of the same general ellipsoidal shape but usually very much larger. Each of these cells contains a bundle of many raphides—long acicular crystals of calcium oxalate inclosed in a mucilaginous sac (figs. 46, 47). Raphides cells are formed very early in the differentiation of tissues within the stem apex (fig. 21).

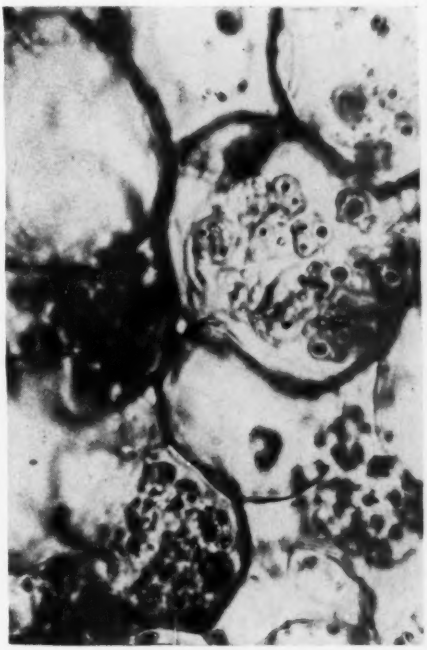


FIG. 44.—Compound starch grains in cells of stelar region in mature portion of stem (cut transversely). Hilum shows up distinctly as dark spot within individual grains, since it is highly refractive to light.

THE CORTEX

Although there is no differentiation into a nodal and an internodal region in

the stele, such differentiation is found in the outer cortex.

The greater volume of the cortex is made up of an inner cortex, the ground tissue of which consists of parenchyma cells similar in shape and orientation to, but smaller in diameter than, those found

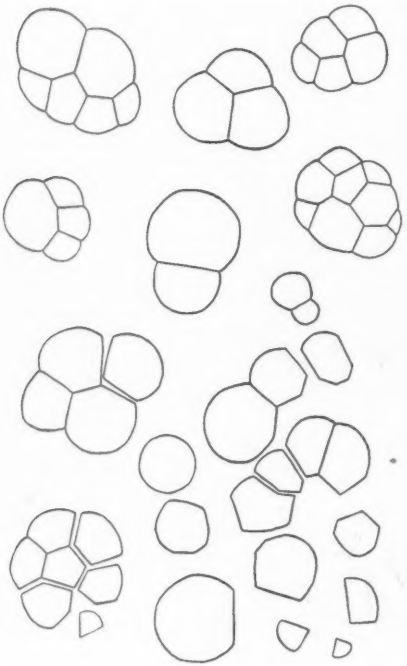


FIG. 45.—Various shapes of compound starch grains and their component parts—simple grains—in stelar region of mature portion of stem.

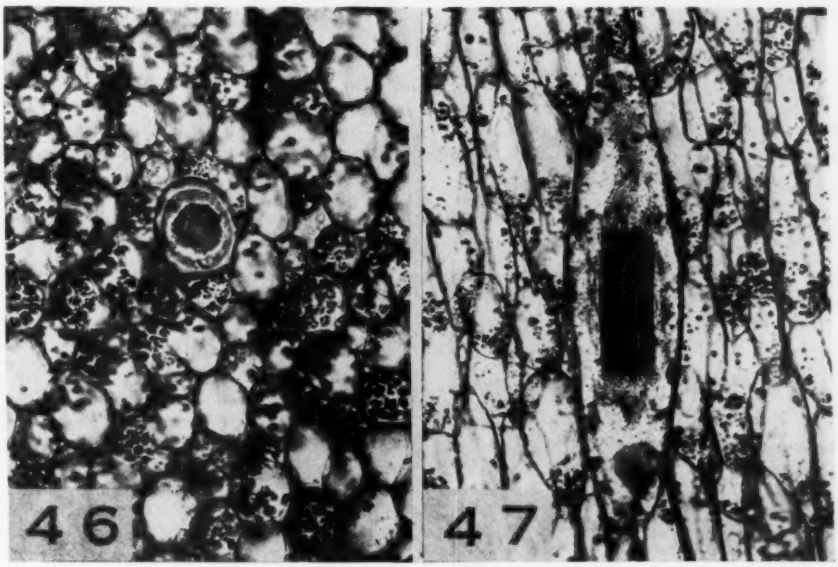
in the stele (figs. 42, 43, 49). These cells contain starch grains of the same type as those found in the stelar cells. Raphides-bearing cells also occur in the inner cortex; these resemble those found in the stele.

External to the broad zone which forms the inner cortex is a narrow peripheral zone, the outer cortex, which consists of seven or eight rows of cells inside the epidermis (figs. 48, 50). In the mature stem the outermost row of this outer cortex, immediately adjacent to the row of epidermal cells, consists of sclerenchyma cells and will be called the *peripheral sclerosed cortical layer*. The use of this terminology in preference to the long-established word "hypodermis" is justified below.

Once the earlier conception of a multiple epidermis—the external layer of the stem (and of the root and leaf) and the layers immediately below it—was superseded by the terminology of "epidermis" and "hypodermis" to designate the same tissues, there seemed to be no question as to the correctness of the use of the term "hypodermis." Several authors (**30, 114, 149**) started the mode in the latter part of the past century, and the term is still being used in general anatomical literature (**33, 60**) and in descriptions of the Bromeliaceae (**13, 141**), where this layer is so pronounced. The term "hypodermis" came to mean, in the stem, root, or leaf, a layer of supporting or protecting cells of any type which lies immediately under the epidermis and reinforces this layer in some way (**33**). As ENGARD (**37**) has finally pointed out—and rightly so—the hypodermis thus described refers to *spacial* (spatial) relationship with the epidermis and not to *ontogenetic*[12] relationship with the cortex. ENGARD has suggested the term "cortical sclerenchyma" for this tissue, but this is so general as to include the sclerenchyma caps on the phloem side of the bundles. MEZ (**93**), in describing another species in the Bromeliaceae, wrote of a "sclerosed layer" beneath the epidermis. This terminology has been combined with that of ENGARD to make the peripheral sclerosed cortical layer suggested by this writer.

[12] ENGARD wrote specifically of the roots of orchids, but the same reasoning is applicable in describing the stems of plants.

The cells of this layer do not mature until quite late in the development of the stem. In the mature portions of the stem it consists of two rows of cells distinct in type from those adjacent to them. The cells inwardly adjacent to the sclerosed layer are transitional in type between these and the cells forming the inner cortex. The cells forming the sclerosed layer and those of the transitional type make up the outer cortex. The cells in the sclerosed layers differ in the nodal and internodal regions.



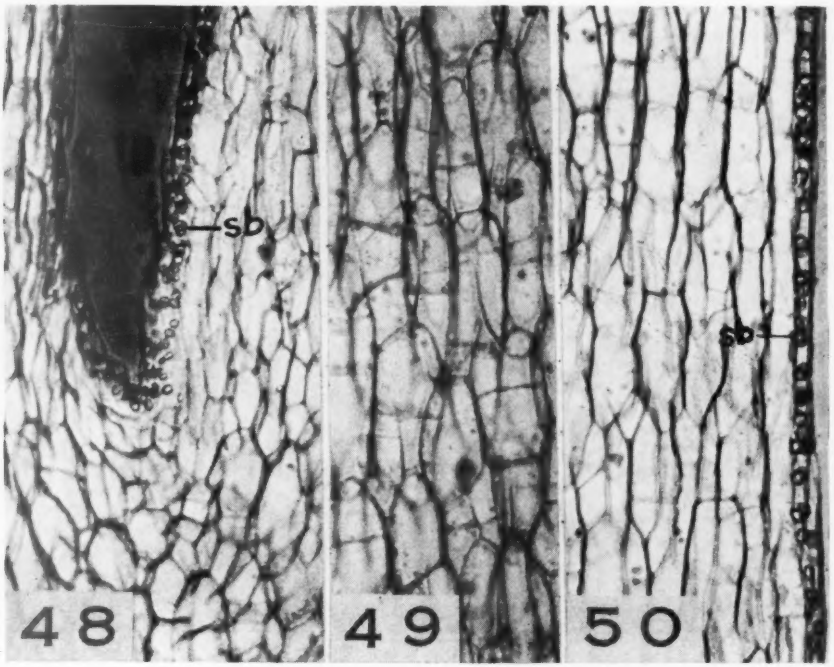
FIGS. 46–47.—Raphides. Transverse, fig. 46, and longitudinal, fig. 47, sections of cortical region of mature portion of stem to show raphides cell. Black spots in remainder of parenchyma are starch grains. Notice mucilaginous sac surrounding raphides.

In the internodal region the mature cells of the outermost row of the sclerosed layer are narrow elongated thick-walled cells—sclerenchyma—with the greater diameter in the direction of the stem axis. They are arranged in regular longitudinal rows. The end walls are usually oblique, or even tapering in some cases, but may often be truncate. In transverse sections of the stem these cells appear as four- to six-sided polygons. Their walls are extremely thick and are profusely pitted; the pits are set obliquely and are of the simple type. In the basal, older portion of the stem the walls are suberized.

The cells in the row adjacent to the one just described—the inner row of the sclerosed layer—resemble those of the outer row in shape, being narrow, although not so narrow, and elongate, but without the thick walls. Instead the walls are not much thicker than those of the adjacent cortical cells, although they are pronouncedly thickened at the corners, collenchyma-like. These cells are not, however, true collenchyma cells. Cells very similar to those in the sclerosed layer and those beneath it are illustrated

in the section describing the subdermal cells in the leaf.

In shape the cells in the next inwardly lying rows pass gradually over from the narrow elongate type to the typical parenchyma cells of the inner cortex. All tions cut parallel to the surface the cells appear as regular or irregular polygons, oriented with their long axis in any of several directions; or, in such sections and in sections cut at right angles to the axis, they are isodiametric. As BERGMAN



FIGS. 48–50.—Longitudinal sections of immature portion of stem to show epidermis and cells of outer (figs. 48, 50) and inner (fig. 49) cortex. Fig. 48, outer cortex at nodal region (portion of leaf on left). *sb*, silica body.

the cells of the outer cortex are without starch grains.

In the narrow zone above the insertion of the leaf, with which the leaf base forms the node (fig. 8), the sclerosed layer consists of a single row of cells, with the rest of the outer cortex made up of one or more adjacent rows of the transitional type of cells described above. The shape of the cells of the sclerosed layer in this region is very diverse, and there is no regularity in their arrangement. In sec- and WELLER (13) have stated, the juxtaposition of these groups of cells to one another produces a high degree of irregularity not found elsewhere in the stem. The cells forming the sclerosed layer in the nodal region are considerably larger in diameter than those in the internodal region.

The cells in the transitional zone between node and internode show transitional forms between those in these two regions.

Eventually the structure of the outer cortex is modified by the formation of cork tissue; this will be described in a later section.

Passing through the inner ground tissue of the cortex are: numerous isolated vascular bundles which are leaf-trace bundles; circumferential bands of vascular tissue just beneath the surface of the stem and a short vertical distance above and opposite the insertion of each leaf (in the nodal region of the cortex); and the adventitious roots. Although each of these differentiated tissue complexes or organs are conspicuous features in the cortical region, they are to be described separately in appropriate sections.

The epidermis

The epidermis of the stem shows the same relationship between form and arrangement, and location in the nodal and internodal regions, as do the cells in the peripheral sclerosed cortical layer.

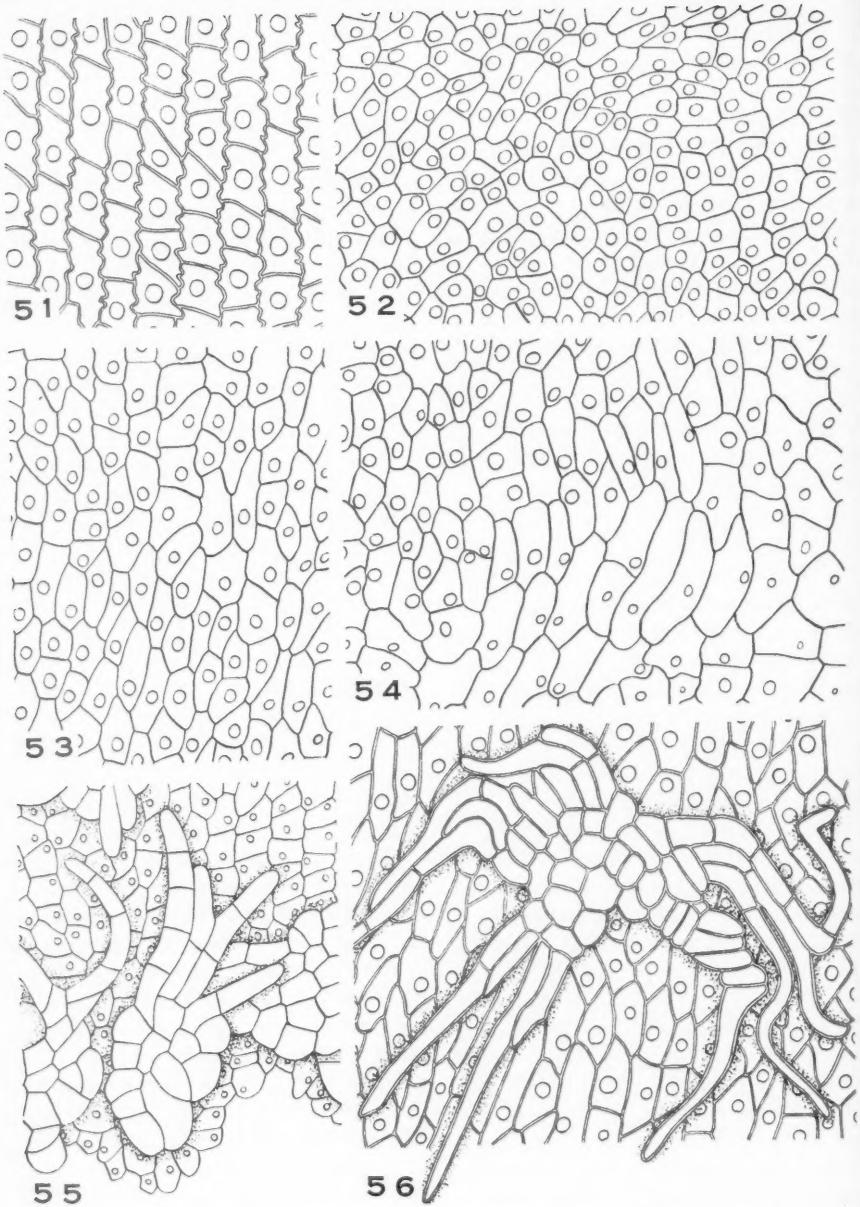
In the internodal region the epidermal cells are usually rectangular or frequently even square as seen in surface view, with the longer dimensions in the first case parallel to the stem axis. The cells are definitely arranged in vertical rows. The walls, primarily those parallel to the stem axis, are usually sinuous, a characteristic of most mature pineapple epidermal cells (fig. 51). In the mature cells the inner and radial walls, especially the former, are much thicker than the outer wall. Each cell contains a silica body (fig. 51), another characteristic feature of the epidermal cell in the pineapple and in most Bromeliaceae. A layer of cutin covers the entire surface of the epidermis in the older parts of the stem.[13]

[13] A more detailed description of epidermal cells in the pineapple plant will be given in the section on the leaf epidermis, in which the same features are to be found but in a more pronounced manner.

In the nodal region the epidermal cells are very irregular in shape and arrangement. They are ellipsoidal, very irregularly polygonal, or even isodiametric in a surface view. In the two former cases the greater diameter may be in any of several directions. These epidermal cells also contain silica bodies, but these are usually of a smaller diameter than those in the internodal cells. The cell walls are smooth and not sinuate, as in the case of most internodal epidermal cells (figs. 52, 54). The inner and radial walls become greatly thickened as in the internodal cells.

The cells in the transitional zone between node and internode show transitional forms between those in the two regions and have smooth walls (fig. 53).

An interesting feature of the surface of the nodal region of the stem is the usually single or sometimes double horizontal row of closely set peltate trichomes located just above the "junction" of each leaf and the nodal region above it (figs. 55, 56). These trichomes form soon after the development of the leaf primordium. They consist of a foot and an expanded head portion. Their development is similar to that of certain isolated trichomes on the leaf and is described in detail in connection with the latter. The heads of the trichomes on the stem node are not of the scutiform type usually found on the leaves and considered typical for *Ananas comosus*, but, because of nonconcentric division and growth of their cells, the form is diverse and irregular. Usually the margin of the heads is fimbriate, the peripheral cells forming long narrow processes (figs. 55, 56). These nodal trichomes develop rapidly and mature before the trichomes on the upper surface of the adjacent leaf are formed. They soon become colored a yellow or yellowish brown.



FIGS. 51–56.—Stem epidermis. Surface views in internodal (fig. 51), in nodal (figs. 52, 54), and transitional (fig. 53) regions. Fig. 55, double and, fig. 56, single row of trichomes on lower part of nodal region. All epidermal cells contain silica bodies.

Occasionally isolated trichomes are found in scattered positions in the upper part of the nodal region; these are either of the same type just described or of the more regular form found on leaves. Trichomes are usually not found in the internodal region.

Stomata do not occur in either the nodal or the internodal regions of the stem.

The vascular system

The vascular system in the pineapple stem is extremely complex, as it is in many monocotyledonous plants; and, although one may assume that a definite system of arrangement exists, it is impossible at this time to draw a diagram of this system, as has been done for so many dicotyledonous plants. Work will continue on this phase of pineapple anatomy, and it is hoped that eventually such a diagram will be available. That this system is complex is not surprising. The bundles are crowded, with approximately eight thousand common bundles (which are leaf-trace bundles for about eighty leaves) distributed throughout the stem, many more bundles making up the branch traces for the eighty lateral branch buds, in addition to the vascular tissue issuing from the bases of the many adventitious roots found in the stem cortex, and the vascular plexus which serves as an intermediary between the leaf-trace bundles and the vascular strands from the roots.

The vascular system of the stem consists, then, of the common bundles which are the leaf-trace bundles and the branch-trace bundles, the vascular cylinder lying between the cortex and stele, and the root vascular system. Although these parts of the system are all intimately interrelated, they form rather definite units and are treated separately: the branch-trace bundles in connection with the lateral branch buds, the root vascular tissue in the description of the root, and the vascular cylinder separating cortex and stele in a separate section (p. 190). This section will describe the common bundles which are leaf traces.

In an attempt to discover some order where at first glance there appeared only complete disorder, it was found that the common bundles which form the traces for any one leaf could be divided roughly into three groups which will be designated as I, II, and III according to the order of their origin and development and their consequent course in the stem.

I. The stem bundle whose prolongation forms the central bundle of each leaf is the first to develop. Soon after a leaf primordium has been initiated in the terminal meristem of the axis a procambial strand is formed in this terminal stem tissue. This strand continues into the leaf primordium as the central procambial strand (figs. 20, 22, 24). Since this first bundle of the latest-formed leaf primordium develops in the portion of the apical meristem of the stem described (p. 186) as the summit of the dome of apical meristem, its course in the stem is principally transverse and gently oblique (figs. 20, 22, 24). The large bundles in the center of more mature portions of the stem belong to this category.

II. Subsequently formed procambial strands, continuing as leaf-trace strands in this leaf primordium, originate after the next successive leaf primordium is initiated. In the interim between the initiation of this youngest leaf and the now second youngest leaf primordium, the latter is moved back from the apex. In this second youngest leaf the procambial strands which now originate laterally to the central strand described above are prolongations of procambial strands still

originating and developing in the terminal apical meristem of the stem, but on the inside of the slope of the meristematic dome, so that their course is less transverse and more oblique. Strands formed subsequent to these, continuing into the same leaf, develop at distances further and further from the center of the

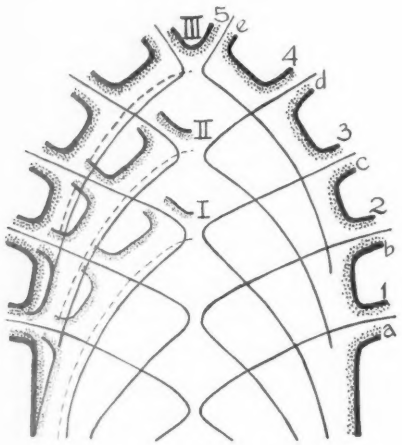


FIG. 57.—Schematic drawing showing course of typical leaf trace bundles in monocotyledonous stems, adapted from ARBER (2). On left side, progressive development of leaves (numbered *1–5* on right side) and corresponding leaf traces (*a–e* on right side) followed through three stages (I–III). These are bundles differentiated within conical sheath of meristematic tissue between cortex and stele, indicated by broken line. On right side, peculiar curvature of bundle course as result of this type of development.

stem, since the meristematic tissue is pushed toward the periphery of what is to constitute the stele; this meristematic zone in which procambial strands are found becomes an ever narrower zone as the central tissue of the stem is differentiated. These later-formed bundles follow a course more and more steeply oblique as the slopes of the meristematic dome become steeper. It is the bundles in this category (II) which have the peculiar inward and outward curvature of monocotyledons, first described by VON MOHL (101) and SCHOUTE (124), and for the first time successfully illustrated by ARBER (2). The inward curvature is the result of the procambial strand conforming to the curvature of the inner side of the hollow dome of meristematic tissue in which it is laid down. The outward curvature is the result of elongation of the stem accompanied by an increase in diameter of the stem. This curvature in the pineapple stem is similar to that illustrated in figure 57.

III. The last-formed bundles of the leaf are initiated between earlier-formed ones and those which finally develop at the margins of the leaf; they are, in most cases, projections of bundles formed in the cortex. These cortical bundles usually anastomose with stelar bundles as the latter pass through the cortex.

This recognition of a difference in course of bundles dependent upon time of development helps in a broad interpretation of the vascular system in the pineapple stem but gives no details as to the modifications in the course of individual bundles incident to the leaves arising in spiral succession and to other variations. These details can be worked out only after a great deal more study.

A little may be said about the course of individual bundles and their structure. To facilitate some of the following descriptions of bundle courses, a series of three-dimensional drawings shows some of the various courses (fig. 58). The first-formed bundle of a leaf passes through the cortex and a leaf gap in the vascular cylinder between cortex and stele at an angle but proceeds toward the center of the stele in an almost horizontal plane owing to its origin (already described). At the center of the stele it anastomoses with similar bundles from other leaves. In a radial section of a stem these bundles

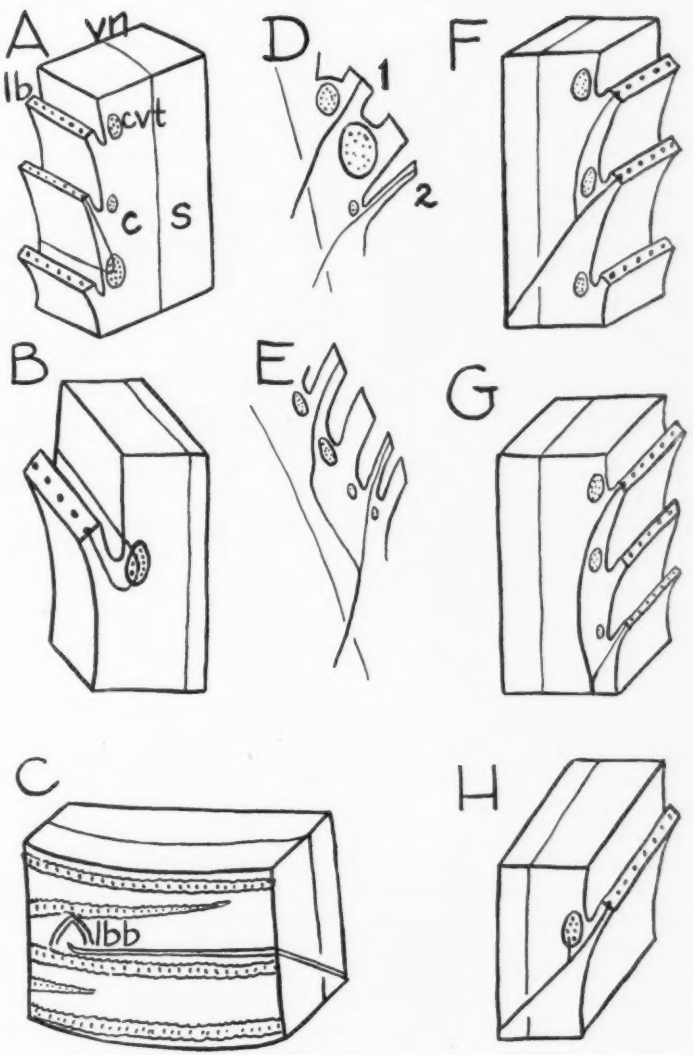


FIG. 58.—Three-dimensional drawings of stem sections to show courses of individual bundles. These are described in the text, pp. 202 ff. *lb*, leaf base; *cvt*, circumferential band of vascular tissue; *vn*, vascular network at boundary between cortex (*c*) and stele (*s*); *lbb*, lateral branch bud.

in this part of the stem are therefore cut in cross section (fig. 10*A*).

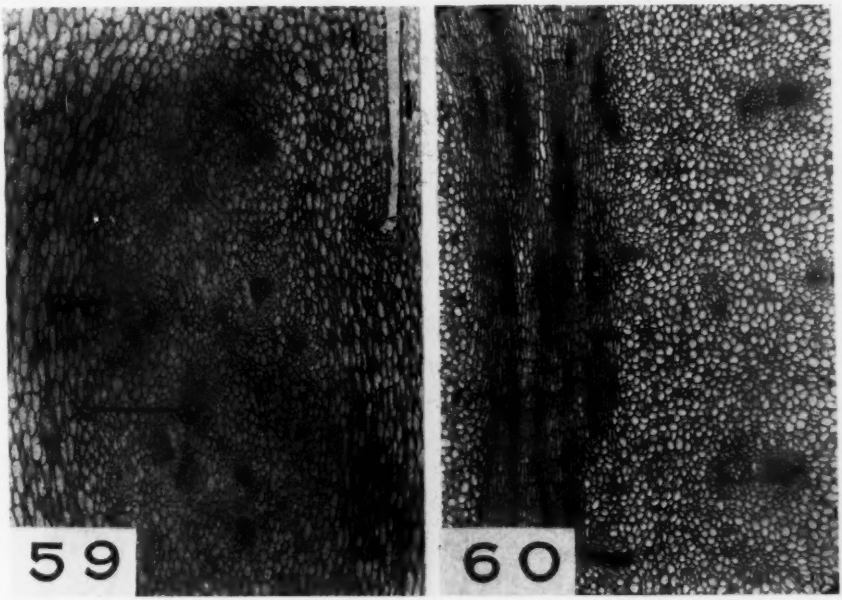
The larger of the median bundles in the leaf, lateral to the one just described, pass through the cortex into the stele at an angle of about 45°, through leaf gaps (fig. 58*D*1), and continue still obliquely to the center or near the center of the stem, before turning downward and outward in a broad curve toward the periphery of the stele. As the lower ends of these bundles approach the network of anastomosing vascular strands which form the vascular cylinder at the periphery, they continue for some distance downward, almost parallel to it, before actually approaching nearer and anastomosing with its elements. Although these large median bundles sometimes follow the course just outlined entirely isolated, the usual thing, as is characteristic for Bromeliaceae (**40, 154**), is for such bundles to anastomose with bundles from other leaves, in either the cortical or the stelar region (figs. 58*E*, *F*, *G*). Such a median bundle, on leaving the leaf, frequently anastomoses in the cortex with a median bundle from the leaf in the spiral turn below before the consolidated bundle enters the stele (fig. 58*F*), or two marginal bundles from leaves in two successive spiral turns will first anastomose with each other and the combined vascular elements from these two join a median bundle from a leaf in the next turn below the second of the two leaves just mentioned. After this second anastomosis in the cortex the consolidated bundle enters the stele. In its curved course through the stele this consolidated bundle usually anastomoses with one or more bundles.

The larger of the marginal bundles of the leaf may enter the stele directly (fig. 58*D*2), but more frequently they anastomose in the cortical region with a median bundle from a leaf in the next turn below (described above) or, even more frequently, at a much lower level. BERGMAN and WELLER (**13**) wrote of these bundles distributed through the cortex as constituting a bundle system distinct from those of the central cylinder, since they do not enter the stele until after they have united with another leaf-trace bundle in a lower part of the stem. These authors also wrote that, before these lateral leaf traces take their downward course through the cortex, they "turn upward through a distance not greater than the thickness of the leaf, deflect laterally for a distance of several millimeters and then (proceed) downwards." This is true for most but not all the marginal bundles, and is also true for some of the smaller median bundles; in other words, most of the later-formed bundles show this deflection. Those portions of the bundles which are deflected are part of the circumferential bundles described as occurring in the nodal region of the cortex (p. 174 and figs. 58*A*, *B*, 59, 60). The other bundles in the circumferential band of vascular tissue are branch traces; these will be described in the next section in connection with the lateral branch buds.

Differentiation within the procambial strands, once they are formed in the apical meristem, proceeds rapidly. The protoxylem is the first part of the bundle to become differentiated; the first elements of this tissue to be formed are the annular cells, with spiral cells appearing later. The formation of the metaxylem follows very closely upon that of the protoxylem; it consists of scalariform tracheids and scalariform- and reticulate-pitted porous vessels, i.e., vessels with scalariform- and reticulate-pitted walls and simple perforations. In the fully developed bundle the xylem portion con-

sists of the protoxylem made up of the remnants of the ruptured annular and spiral cells and the resulting protoxylem lacunae—these may be very small or even absent in the stem bundles, in contrast to the large lacunae found in the bundles in the leaf; the metaxylem, consisting of a varying number of scalariform tracheids and scalariform- and reticulate-pitted vessels which are arranged rather irregularly as seen in cross sections of the bundles; and the accompanying parenchyma (figs. 61–64). Variations of the above described tissues in various regions of the stem will be presented later.

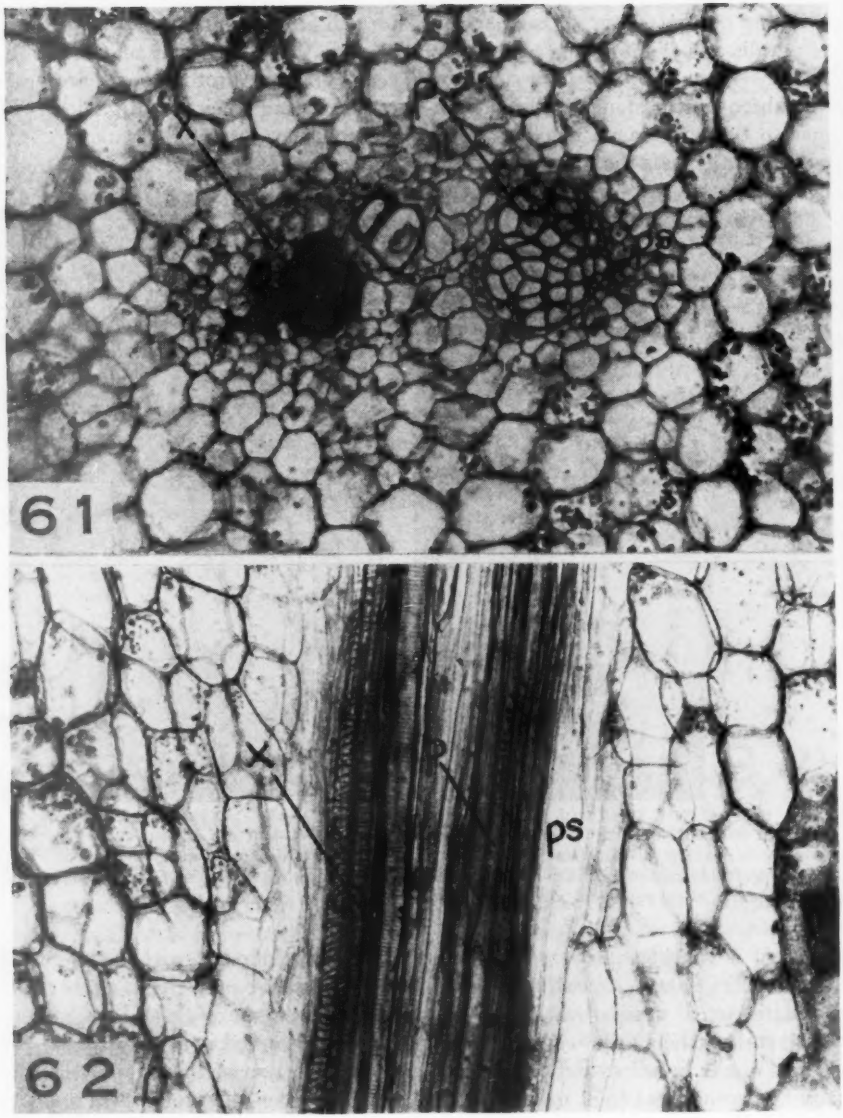
The protophloem is differentiated after the protoxylem; its cells differ little from the procambial cells from which they are derived. The metaphloem is formed after the metaxylem and consists of sieve tubes and companion cells. The cells of the protophloem are early crushed, and their identity lost. The metaphloem forms a circular or oval figure in cross section (figs. 61–64).



FIGS. 59–60.—Fig. 59, longitudinal section of peripheral portion of stem near leaf (*l*) base to show cross-sectional view of circumferential band of vascular tissue, consisting of deflected leaf traces and axillary bud bundles. Fig. 60, transverse section of stem in same portion of stem to show longitudinal view of band. *in*, internode, and *n*, node; *p*, phloem, and *x*, xylem portions of bundles in band.

The development within the bundle described above proceeds in a centripetal manner, the metaxylem and metaphloem each being formed from the adjacent procambial portion of the strand which remains between the protoxylem and protophloem. In the mature bundle the xylem and phloem portions are usually separated by a row or two of parenchyma cells (figs. 61, 63).

Adjacent to the phloem side of the bundle a group of narrow elongate thin-



Figs. 61–62.—Cross (fig. 61) and longitudinal (fig. 62) sections of vascular bundle in stem. *x*, xylem, *p*, phloem elements; *ps*, parenchyma sheath cells. Individual elements in bundle described in text (pp. 204 ff.).

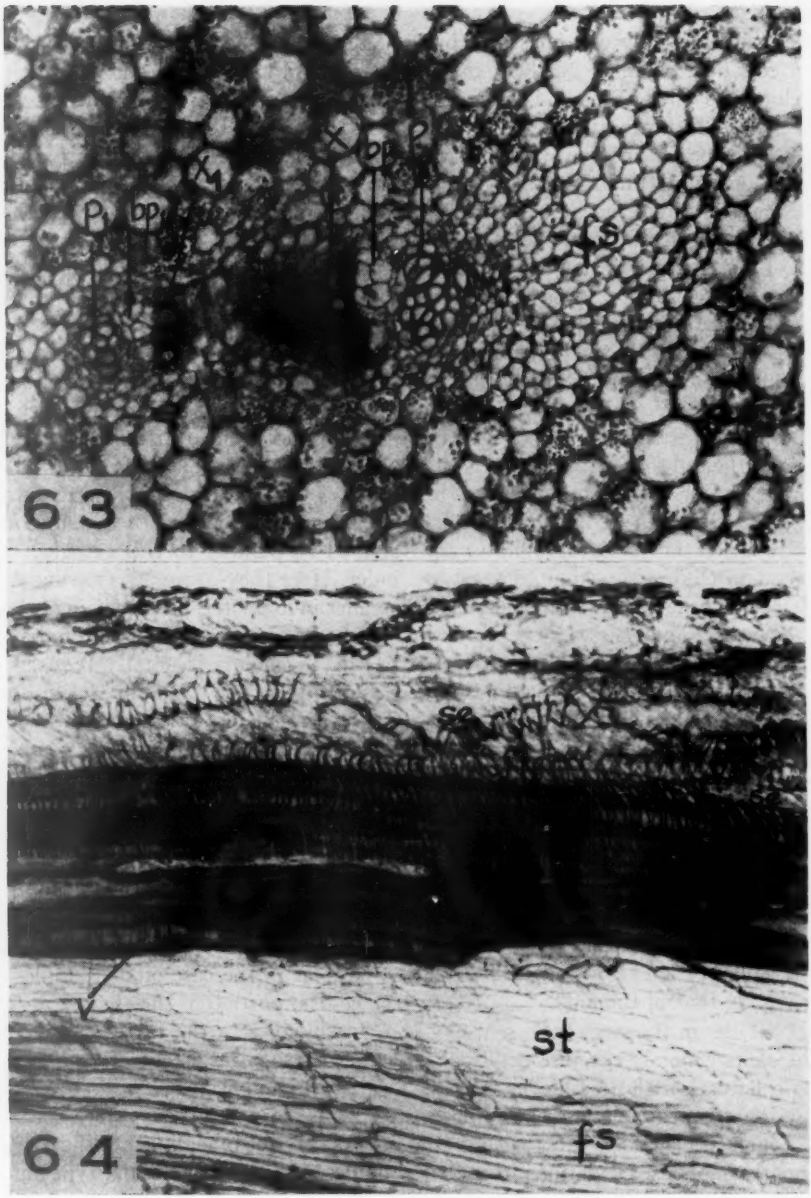
walled cells are early differentiated, but these cells do not mature until late in the development of the stem. These cells, when mature, form a crescent-shaped fibrous subsheath or "cap" which is of great mechanical importance (figs. 63, 64). The cells are sclerenchyma and when mature are dead and without a protoplast. In cross section they are irregular polygons with four to seven sides. The walls become greatly thickened; often they are so thick that the lumen may be but a mere channel through the center of the fiber. The thickening layers on the inside of the walls are deeper at the angles of the cell so that the lumen is circular or oval in cross section instead of angular. The lumen is divided at intervals by septa. These dividing plates are thin and of cellulose, in strong contrast to what become the heavily lignified or suberized walls. That these are septa and not cross walls is shown by the lack of secondary walls corresponding to those on the lateral walls and by the fact that the middle-lamella-like septum does not extend to the middle lamella of the side walls but ends at the surface of the secondary wall. The lateral walls are profusely pitted in contrast to the cells which form the fibrous sheaths of the vascular bundles in the leaf. The ends of these sclerenchyma cells taper gradually, the tapered portion of one adjoining cell overlapping that of the next.

All bundles in the stem are essentially of the same general type just described, being collateral, with the phloem toward the periphery of the stem, except in those cases where the bundles are twisted, as frequently happens. Deviations from the more normal structure and orientation will be noted in the following descriptions of portions of bundles in different regions of the stem.

The bundles cut in the center of the stem are the largest and most widely spaced. As stated previously, these are the first-formed leaf traces. In cross section these bundles are greatly elongated ovals or ellipsoids. Frequently the sclerenchyma cap on the phloem side is immense (fig. 63). Two or more of these bundles anastomose to form a large consolidated mass of vascular elements (fig. 65). In this case there is a juxtaposition of the xylem and phloem elements from the anastomosing bundles and, consequently, a deviation from the normal relative positions of these two tissues.

The bundles cut near the periphery of the stele constitute the lower ends of many of the leaf-trace bundles. These are cut in that portion of their course described as parallel to the vascular cylinder. Here they are more crowded and smaller in diameter, usually being nearly circular in cross section. The number of vascular elements is greatly reduced in this portion of the bundle; it has but one to four tracheids and eight to twelve sieve tubes and accompanying companion cells. This portion of a bundle is, furthermore, without the large fibrous cap described for the large central bundles. In more mature portions of the stem, however, these small peripheral bundles may have either a small or a large fibrous cap on the phloem side, although never so large as in the case of the bundles in the central portion of the stem.

The bundles in the cortex closely resemble those in the stele; with the exception of the bundles wholly cortical, they are continuations of the same vascular tissue. Near the inner limits of the cortex, bundles of the smaller circular type found at the periphery of the stele are present (figs. 66, 67). There is frequent anastomosing of bundles in the cortex, as already mentioned; in this case there is juxtaposition and consolidation of ele-



FIGS. 63–64.—Cross (fig. 63) and longitudinal (fig. 64) sections of vascular bundle shown anastomosed with smaller bundle. *p*, phloem; *x*, xylem; *bp*, parenchyma cells in bundle separating xylem from phloem in larger bundle; p_1, x_1, and bp_1, are same tissues in smaller bundle. *fs*, fiber sheath on phloem side of bundle; *se*, spiral elements of xylem; *st*, sieve tubes of phloem; *v*, vessels.

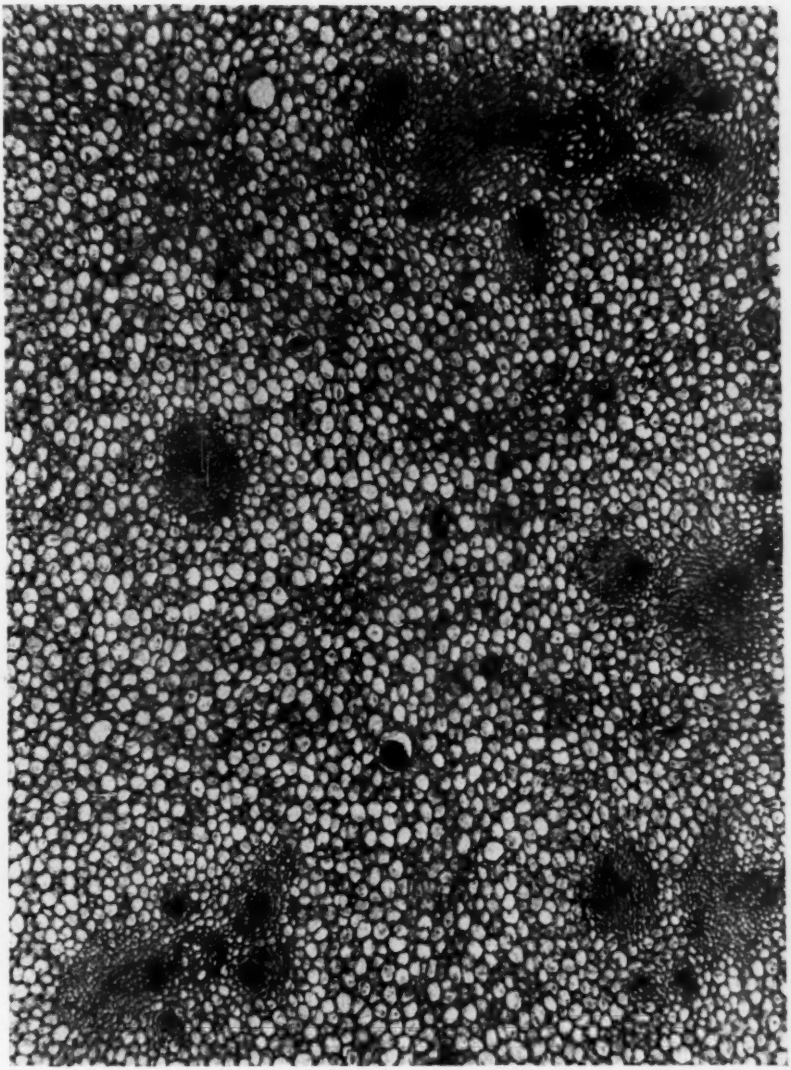
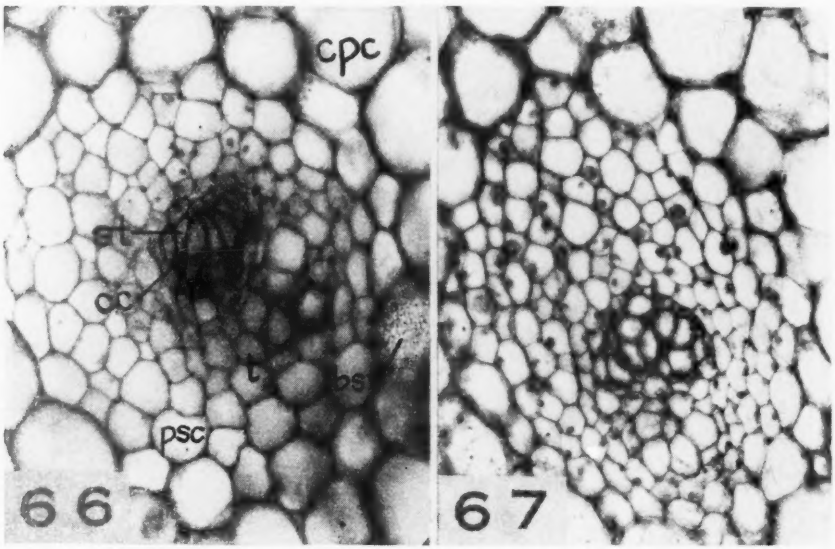


FIG. 65.—Transverse section of immature portion of stem to show anastomosing bundles (leaf traces)

ments as described for such bundles in the stele.

In the more mature portion of the stem those bundles, both large and small, cut in both cortex and stele showed an endodermal sheath around the xylem portion of the bundle. The cells forming pitted, the pits being simple, with the apertures tending to be elliptical in outline in face view. The longer axis of the pit aperture is usually oblique. The walls of the cells become lignified. These endodermal sheath cells are illustrated in the section on leaf bundles.



FIGS. 66–67.—Cortical bundles. Cross sections of bundles in inner cortex at early stage of development: *st*, sieve tubes; *cc*, companion cells; *cpc*, cortical parenchyma cell; *psc*, parenchyma sheath cell; *bst*, same, with tannin. In fig. 67, bundle has only phloem cells (sieve tubes and companion cells).

this sheath were not recognized as such by this writer until similar endodermal sheaths were found in the bundles in the leaves. There they are more distinct and surround both the xylem and the phloem.

The cells of this endodermal sheath in the stem bundle are about one-third as long as those in the fibrous cap and with a somewhat greater cross-sectional diameter. In cross section these cells form polygons. The walls are thick when the cells are mature but not so thick as those of the fibrous cap cells. The walls are

The lateral branch, or axillary, buds

In the pineapple a bud of a lateral branch, or axillary shoot, occurs in the axil of each leaf. Such an axillary bud is situated in the median plane of its subtending leaf. In a plant of the age under consideration—just before floral differentiation—the development of these buds is in an arrested state. After floral differentiation, growth of many of them is resumed, although only a small number of the total formed, and usually only those near the apex of the stem, develop into full branches capable of bearing

fruit; the remainder are then considered dormant buds.

The buds are greatly flattened as a result of being tightly appressed between leaf base and stem. Frequently a depression in the leaf occurs (fig. 68*G*); this resembles the bud furrow or eye groove in the internodal region of the sugar cane stem (**5**). This depression is no doubt a result of the pressure of the bud on the young tissue of the basal portion of the leaf during its development.

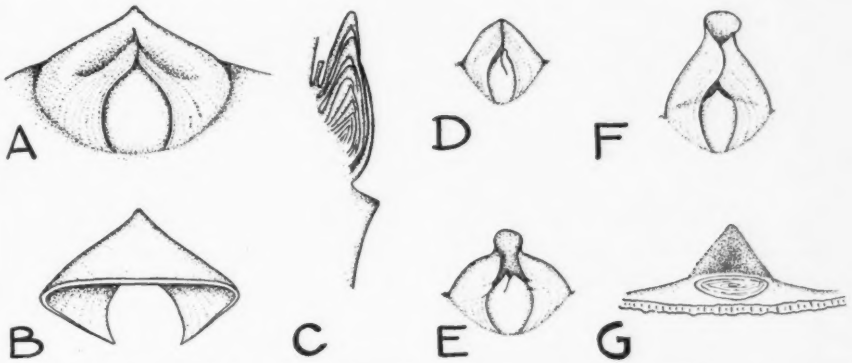


FIG. 68.—Lateral branch buds. Variation of bud types on different parts of stem: *A*, most typical type of bud; *B*, prophyll removed to show form; *C*, longitudinal section of most typical type of bud; *D*, bud near apex; *E*, bud near base; *F*, bud on central part of stem; *G*, leaf removed and bud cut off at base to show bud furrow.

The buds are smallest in the basal and apical portions of the stem; in the first case, because their growth is arrested at an early stage and because they remain as such during the lifetime of the plant unless otherwise stimulated;[14] in the latter case they occur near the growing region of the stem and are thus in an early stage of development. The smaller buds near the apex of the stem are ovate in shape, with blunt-pointed apices[15] (fig. 68*D*). The height of these buds from base to tip is approximately 3–5 mm., and the width at the base about 5 mm. The buds near the base of the stem are about the same size, but the tip of the prophyll is open and flared (fig. 68*E*) instead of blunt-pointed.

On the central portion of the stem, where the buds are largest, they are elongated, with the tip of the prophyll open and flared (fig. 68*F*). The height is about 2 cm., and the width at the base about 1 cm. These buds are especially characterized by the fact that their greater size—height—over that of the basal and apical buds is not the result of an actual increase in size of the bud as a whole but only in that of the prophyll.

The prophyll of the more typical branch buds of the pineapple, i.e., those which normally develop into full branches (fig. 68*A*), consists of an anterior or front side composed of two overlapping halves (both right- and left-handed overlapping

[14] The exception to this is the natural but infrequent growth of one or more of the basal buds at or below the soil surface to form a sucker.

[15] The terminology used in the description of the lateral branch bud of the pineapple is for the most part the same used by ARTSCHWAGER (**5**) for sugarcane buds. The buds in the two plants resemble each other in many respects and are of a type rather typical of monocotyledonous branch buds in general, from descriptions by ARBER (**2**).

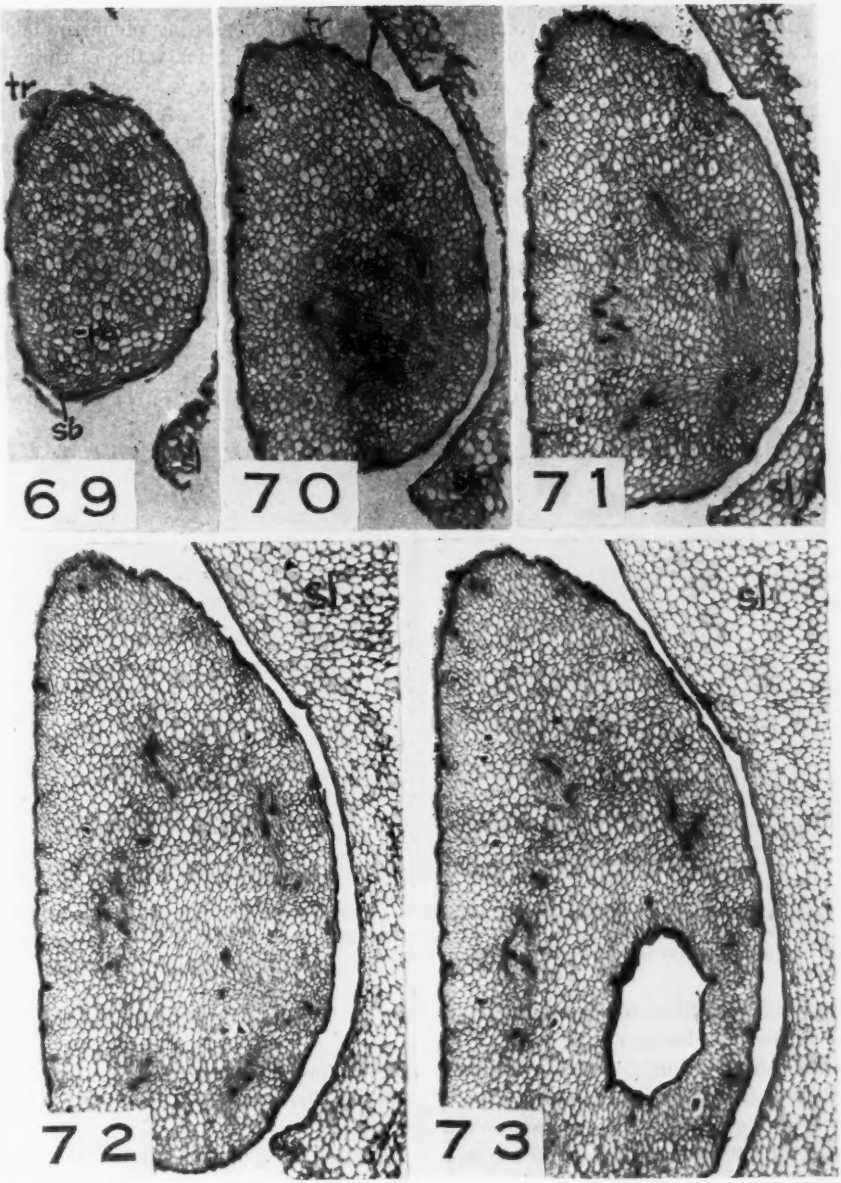
occurs), and the relatively short (in height) posterior side which is entire and basally limited by a curved line which marks its attachment to the nodal tissue. When removed in its entirety, the prophyll forms a kind of asymmetrical hood (fig. 68*B*). The junctures of the anterior with the posterior sides form two keels. There are no definite wings on the bud prophyll of the pineapple—it is without flattened and attenuated keels—as there are on the sugar-cane prophylls.

At the tip of the prophyll, in the region where the two overlapping halves of the anterior side unite, there occurs a kind of opening in the form of a slit. ARTSCHWAGER (5) named this the "germ pore" in the sugar-cane prophyll. This is unfortunate, since this term is already used to describe a pit in the surface of a spore envelope through which the germ tube makes its appearance (67). This structural feature of the prophyll represents, of course, the opening through which the foliar organs of the branch push when growth is resumed.

The edge of the overlapping half of the anterior side is drawn out to form a membranous margin; this extends from just below the tip of the prophyll, from the slit opening just described, downward to its base. This membranous margin does not terminate in an appendage, i.e., as a lateral expansion, as it does frequently in the sugar-cane prophyll. The margin is narrowest near its distal end; it is approximately the same width the rest of its length.

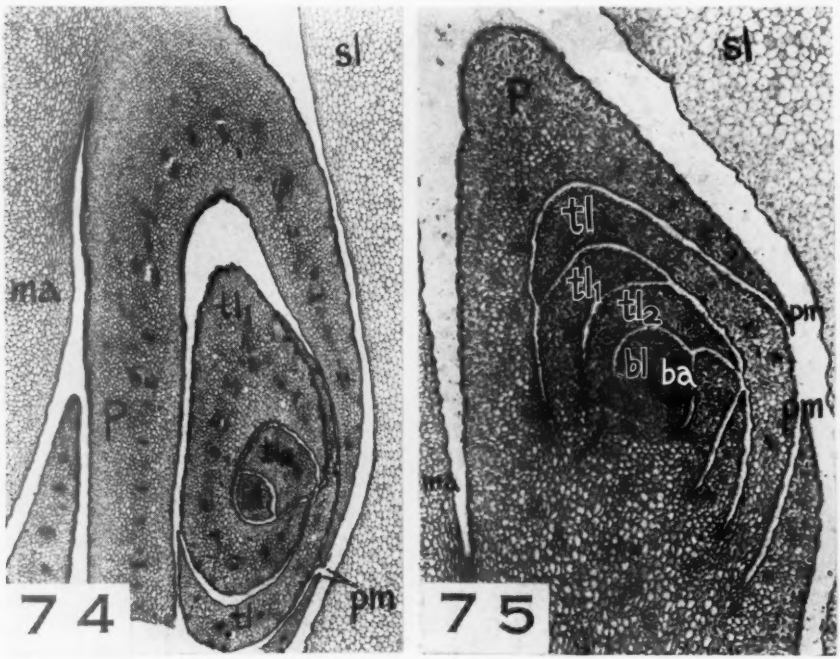
The internal anatomical structure of the prophyll resembles that of a very young leaf or the base of a developing true leaf in almost all respects (leaves will be described in detail in the next section). A transverse section cut through the bud shows that the prophyll has a single row of vascular bundles, larger ones alternating with smaller ones. This row stretches from near the margin of one half of the anterior side, through the posterior side, to the margin of the other half of the anterior side, and is located approximately midway between the two epidermis (fig. 74). There is no distinct median bundle in the pineapple prophyll, as there is also none in the true leaves of the main axis. Some authors have maintained that this two-keeled type of prophyll, with no conspicuous median bundle, represents the fusion of two foliar members; STRASBURGER (146), for example, wrote that in monocotyledons there is a bracteole; this is frequently two-keeled, and, as such, it must be regarded as arising from the union of two bracteoles. ARBER (2) felt that, although this theory appeared plausible at first glance, it does not survive a critical study so well as the older interpretation that the prophyll is a single leaf. According to the latter explanation, the two-keeled form and the lack of a midrib result from the fact that, as the prophyll develops, it is compressed between the main axis on the one side and the axillant leaf on the other. ARBER believed that the most telling evidence in support of this view is the vascular system of prophylls; this is confirmed by a study of pineapple prophylls. The vascular bundles have the same general structure as those of the true leaves but are smaller in cross section and have fewer elements.

Alternating with the vascular bundles are aerating canals, similar to those in the true leaf of the main axis; they have, however, a very much smaller cross section and contain very few stellate cells. A single row of isolated fiber strands, consisting of a few rows of fibers each, occurs between the row of vascular bundles and each epidermis. The ground tissue of the prophyll consists of the type



FIGS. 69–73.—Successive transverse sections through prophyll from near tip to near base, to show internal structure. *ra*, raphides cell; *sb*, silica body; *avt*, anastomosing bundles near tip; *sl*, subtending leaf of bud.

of parenchyma found in the young true leaf and in the basal portion of older true leaves: the cells are subglobose, appearing almost circular in transverse sections and in sections cut parallel to the surface, and more or less elongated in the direction at right angles to the prophyll axis. dermal layer consisting of narrow elongated cells with thick walls, of the type found in true leaves, is also present. Stomata and peltate scutiform trichomes occur on the outer surface of the prophyll —this corresponds to the lower side of the true leaf on the main axis. Trichomes



FIGS. 74–75.—Transverse sections of lateral branch bud to show prophyll (*p*); transitional leaves (*tl*, tl_1, tl_2, in order formed); true lateral branch leaf (*bl*); lateral branch apical meristem (*ba*); subtending leaf (*sl*); main axis (*ma*); margins of anterior side of prophyll (*pm*).

These cells are filled with starch grains. Cells with raphides appear here and there in this ground tissue.

The cells of the epidermis resemble those of the true leaf on the main axis, being square or rectangular in surface view, with sinuous walls. A silica body is found in each of these cells. The walls show the same type of thickening as in the true leaves, the lateral and inner being thicker than the outer walls. A subalso occur on the inner surface of the prophyll; this corresponds to the upper side of the true leaf. Both the stomata and the trichomes resemble those on the true leaves.

The bud proper, i.e., the apical growing-point and leaves of the lateral branch, are inclosed within the prophyll. There is some question whether the next few successive foliar organs inclosed within the prophyll are true foliage leaves, scale

leaves, or bud scales. BERGMAN and WELLER (13), writing of much older branches but of comparable foliar individuals, described a series of small leaves which they called "scale leaves"; these increase gradually in size up to normally developed foliage leaves. The usual interpretation of scale leaves is that of early leaf forms of a shoot such as bud scales, which in turn are designated as the coverings of a bud (67). Both terms would cover the organs under consideration but are certainly not specific. If they are true foliage leaves, they show such modifications in outward form as their development within the prophyll, tightly appressed between leaf base and stem, would bring about—the presence of one or two keels (figs. 74, 75). LONAY (89) called these organs "foliage leaves" on the axillary buds of the monocotyledonous plant he described and ascribed their form solely to pressure. They might best be called intermediate or transition forms between the prophyll and the true foliage leaves. The distinction between bracts or scale leaves and true foliage leaves has never been made very clear; some authors have considered the former as modified leaves, the modification being one chiefly of size. Other authors have written that the internal structure is also different, being simpler than that of the foliage leaf. However, when a foliar organ passes from the category of bract to that of true foliage leaf is not very clear.

In the pineapple axillary bud, the internal structure of the next foliar organs resembles that of other young leaves on the main axis (figs. 68*C*, 74–76).

The apical growing-point of the lateral branch is similar to that of the main axis. The ontogeny of the lateral branch will be included in the description of its vascular system below.

Until the lateral branches expand after floral differentiation of the main axis, their leaves remain prechlorophyllous, and the bud must therefore depend upon the leaves on the main axis for its food supply. Even after the lateral branch elongates and its leaves contain chlorophyll, thus becoming photosynthetic units themselves, the water and mineral supply must come by way of the main axis until the adventitious roots of the branch emerge and are capable of absorbing these materials collected in the axils of the subtending leaves. At best this axillary supply is somewhat limited, and the main axis continues to be the path of mineral and water supply. Because of the rather extended dependence of the lateral branch upon the main axis, the vascular connection between the two is interesting.

In the differentiation of tissue in the apex of the main axis a group of cells in the axil of each leaf remains meristematic (fig. 33). From this meristematic tissue the bud of the lateral branch is initiated (fig. 34). In its earliest stages this resembles the earliest stage of leaf primordium formation in so far that a projection is pushed beyond the surface of the main axis. However, from this stage the two types of primordia differ in that the apical growing-point of the branch bud is, of course, capable of initiating foliar organs; the first of these is the prophyll.

In the development of the branch bud the apical growing-point is far less dominant than in the main axis. There is little elongation of the branch axis, whereas the prophyll and subsequently initiated leaves elongate, if slowly, over a considerable period before growth is arrested (figs. 68*C*, 76). Also, there is not quite the same association between the initiation of a leaf primordium, and the formation

of the first procambial strand in it and in the apical growing region of the branch axis as there is in the main axis. Procambial strands in the bud leaves become prolongations of procambial strands in the stem of the main axis, so that the entire vascular system of the branch bud becomes continuous with the bundles in the main axis. The bundle supply entering the branch is from two sources: a large group of bundles which enters directly from the center of the main axis (fig. 58*C*), and a leaf supply coming from a band of circumferential bundles just below the surface of the main axis and partly just above and opposite the zone of attachment of the leaf on the main axis (figs. 59, 60). The lateral limits of each band are determined by the marginal limits of each subtending leaf, i.e., the

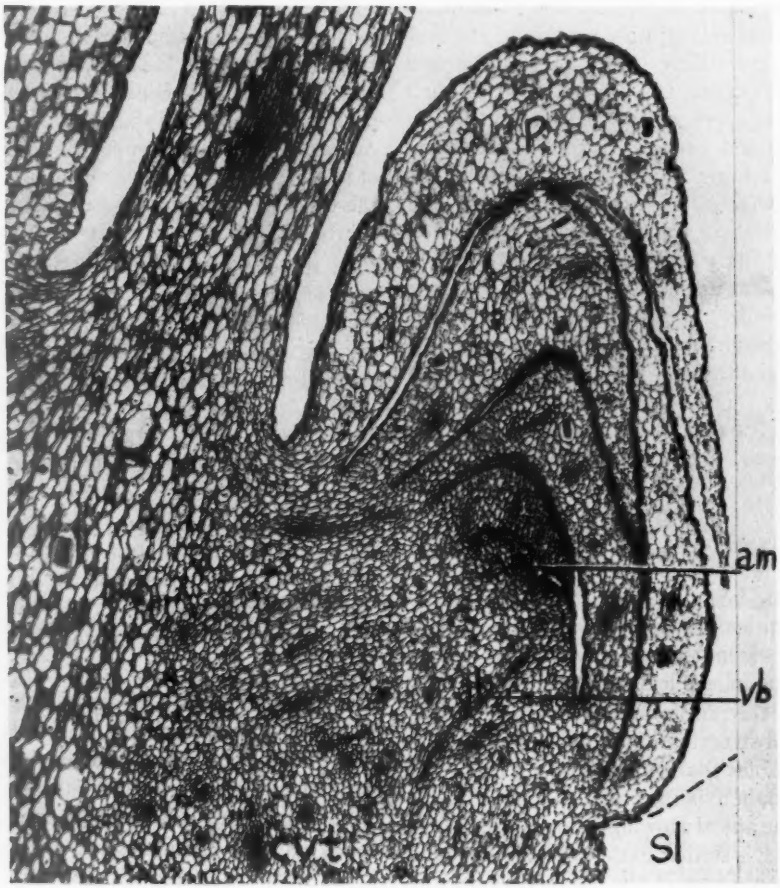


FIG. 76.—Longitudinal section, slightly oblique, of lateral branch bud. *am*, apical meristem; *vb*, procambial strand, entering bud from circumferential band of vascular tissue (*cvt*); *p*, prophyll; *sl*, subtending leaf torn off.

band of vascular bundles extends the same distance around the circumference of the stem as does the leaf inserted immediately below it. This band of bundles is composed of various bundles deflected laterally from their otherwise "normal" course: bundles from the subtending leaf or the leaf above or from various regions of the stele.

After the branch resumes growth, following floral differentiation of the main axis, the usual independent vascular system develops as well.

Before leaving the subject of the lateral branches on the main axis of the pineapple, this writer wishes to comment on the unfortunate use by pineapple field men of the term "sucker" for these branches. Botanically they are shoots and should be called that; a sucker is a shoot of soil surface or subterranean origin.

ANATOMY AND HISTOLOGY OF THE PINEAPPLE INFLORESCENCE AND FRUIT[1]

MARION C. OKIMOTO

Introduction

The collective fruit of pineapple, *Ananas comosus* (L.) Merr., has not hitherto received the anatomical study that its botanical interest and economic importance seem to justify. Taxonomists who have presented brief descriptions of the flower and fruit of pineapple in comparison with those of other members of the Bromeliaceae include BEER (**4**), WITTMACK (**25**), BAKER (**3**), MEZ (**17, 18**), HARMS (**11**), and SMITH (**24**). Of these, WITTMACK, MEZ, and HARMS have considered the general floral characteristics of the family in more detail. Workers in other fields who have described some features of the flower or fruit include HUME and MILLER (**14**), who compared several varieties; KERNS, COLLINS, and KIM (**15**), who traced inflorescence development; and SIDERIS and KRAUSS (**21**), who studied growth phenomena. None of these workers has presented a comprehensive interpretation of morphology and anatomy based upon a study of the vascular system; none has studied the histology of the flower and fruit.

The present study is concerned with the gross morphology, vascular anatomy, and histology of internal structures of inflorescence and fruit. No attempt is made to trace the ontogeny. Smooth Cayenne, the variety commercially grown in Hawaii, was used for this study.

Observations

GROSS MORPHOLOGY OF INFLORESCENCE AND FRUIT

The strobiliform collective or multiple fruit of pineapple develops from numerous sessile flowers that are connate with their subtending bracts and with one another (figs. 1, 2). Its axis, a continuation of the peduncle, is a fibrous stem which contains the many vascular bundles that supply the flowers and bracts

[1] Published with the approval of the Director as Technical Paper no. 177 of the Pineapple Research Institute of Hawaii, Honolulu, T.H.

and which is continuous with the short stem of the leafy crown. Between the uppermost flower and the crown is a transition region with bracts but no flowers. The cortex of the axis cannot be distinguished from the bases of the ovaries and the noncarpellary tissues between them, for all merge with no recognizable lines of demarcation. The edible part of the fruit consists chiefly of ovaries and of the bases of sepals and bracts and, from the morphological viewpoint at least, of the cortex of the axis.

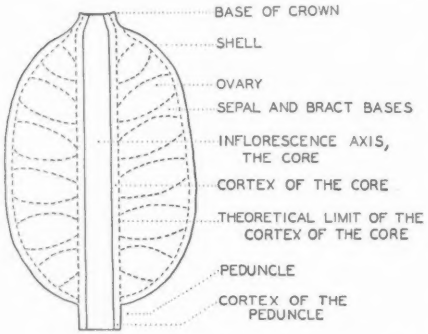


FIG. 1.—Gross morphological structure of pineapple inflorescence.

The firm rind of the fruit, known as the "shell," is composed chiefly of sepal and bract tissues and the apices of the ovaries.

The horticulturally important varieties are parthenocarpic and self-incompatible, but most of them will set seed when cross-pollinated.

The one hundred to two hundred flowers are aligned in three series of spiral rows typical of an $\frac{8}{2}\frac{}{1}$ phyllotaxy in contrast with the foliar phyllotaxy of $\frac{5}{1}\frac{}{3}$ (15). Flowering occurs in acropetal sequence with one to several flowers opening each day during 3–4 weeks, depending on the number of buds present.

FLOWER AND BRACT.—The hermaphroditic flower is trimerous, with three sepals, three petals, six stamens in two whorls of three, and one tricarpellary pistil (figs. 4, 10). Each flower (fig. 5) is subtended and, at its base, half-surrounded by a bract that is thick and fleshy below but tapers to a slender, papery thin tip. Parts of three other bracts complete the enclosure of the flower. The margins of the bract are serrulate below but entire above, and its abaxial surface is clothed with peltate trichomes of the type common to pineapple leaves (19).

The calyx, distinct above and synsepalous at the base (fig. 6), projects vertically beyond the ovary. The sepals, appearing similar to the bract in structure, color, and texture, are deltoid and nearly equal in height. Aestivation is more often convolute than imbricate, but both types are present on the same inflorescence.

The linguiform petals, white at the base and violet or purplish-blue beyond the calyx, are ligulate with two fleshy scales at the base on the adaxial surface (fig. 7). Aestivation is imbricate with the uppermost petal wholly internal. One day after anthesis the petals become soft and pink and begin to twist about one another.

The stamens of the outer whorl are episepalous. The inner, opposite stamens are epipetalous, with each filament base nearly surrounded by (but not fused to) the two fleshy scales of the petal. Stamens of the two whorls are approximately equal in height, shorter than the style, and the petals but slightly longer than the sepals. The fleshy filaments vary in cross section from subcircular to elliptical or sometimes almost triangular. The anthers are introrse, dorsifixed, and two-lobed, each lobe containing two elongate pollen sacs with numerous,

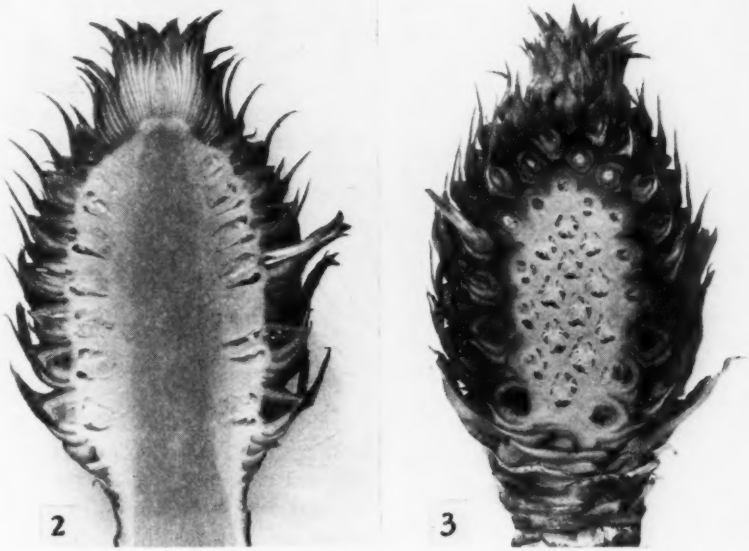
finely sculptured, oval pollen grains. The pollen is released early in the morning by a longitudinal dehiscence along the adaxial side of each lobe.

The three pale-violet stigmas, each $1\frac{1}{2}$ to $2\frac{1}{2}$ mm. long, flare from the top of the style. Each is irregularly linear, flattened and leaflike at the apex, but folded adaxially below, then narrowed until it merges with its lobe of the style. Its receptive adaxial surface is continuous with the lining of the stylar canal.

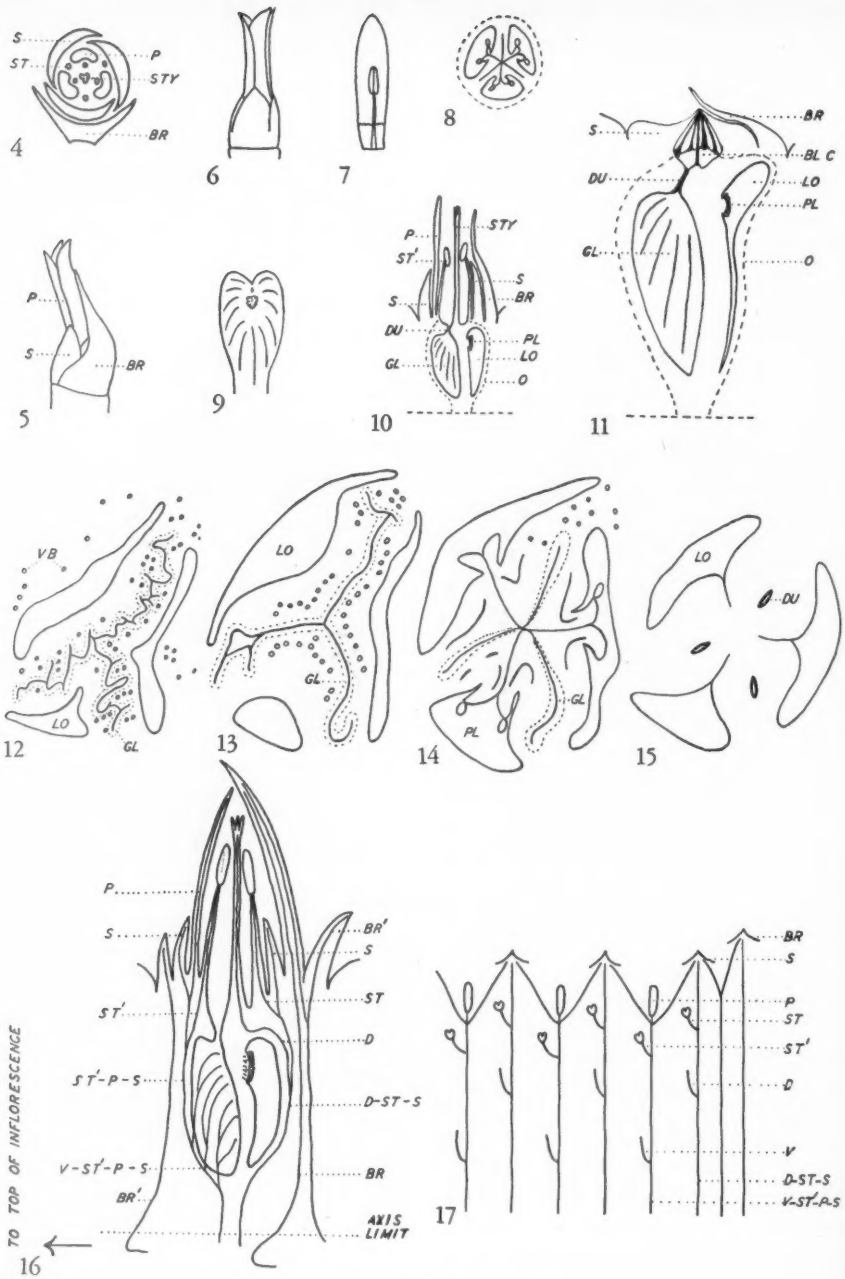
The style, almost as long as the petals, is fleshy and three-lobed, with three distinct stylar canals. At preblossoming and flowering stages each canal is an unobstructed open channel from the stigma to the opening in the locule directly above the placenta. This is easily demonstrated by applying a droplet of India ink to the stigma prior to anthesis.

The inferior ovary is tricarpellate and trilocular, with the three septa forming an inverted **Y** as seen in a tangential section of the inflorescence (figs. 3, 8). Ovaries of adjacent flowers are separated by noncarpellary parenchymatous tissue consisting largely of calyx and bract bases. Limits of the ovary wall cannot be determined precisely because of complete fusion with noncarpellary tissues, but several lines of evidence, including inward extension of effects of toxic chemicals applied to bracts and sepals, indicate that ovary walls are thin and that the bulk of parenchyma between ovaries consists of basal tissues of four bracts (the subtending bract and parts of three others) that completely surround each ovary. The apex of the ovary is cone-shaped.

Usually anatropous but occasionally



FIGS. 2–3.—Inflorescences at middle of flowering with unopen buds above and withered flowers below open blossoms. ×2/3. Fig. 2, median longitudinal section. Fig. 3, tangential section exposing transverse sections of individual flowers. (Photographs by MAMORU ISHII.)



FIGS. 4–17.—Legend on facing page

orthotropous ovules are borne in two rows on the axile placenta near the top of each locule (figs. 8–10). The number ranges from fourteen to twenty per placenta. The ovules ordinarily remain immature and abortive. Seeds which rarely develop except after cross-pollination with other varieties are approximately 3–5 mm. in length and 1–2 mm. broad. The seed coat is brown, coriaceous, and roughened by longitudinal ridges. MILES THOMAS and HOLMES (**19**) have studied the seed structure.

Each pineapple flower normally contains three large internal nectaries or septal glands, one in each septum, which extend almost to the base of the ovary. First described in certain monocotyledons by BRONGNIART (**6**), such glands are now known to occur in several families of the Liliiflorae and Scitamineae (**1, 2, 6, 7, 10, 20**). From studies of the Bromeliaceae by BRONGNIART (**6**), GRASSMAN (**10**), SCHNIEWIND-THIES (**20**), and especially by BUDNOWSKI (**7**), who dealt with at least eighty-nine species, it is probable that septal glands occur universally in this family. BUDNOWSKI reported them in *Ananas bracteatus* (Lindl.) Schultes, a species very close to *A. comosus*, but the presence of such glands in the latter appears to have been overlooked by those working with this fruit.

Each gland consists of an epithelial layer covering a considerable portion of the abutting walls of two adjacent carpels. Ontogenetically, these walls have not fused (**10**), thus leaving a narrow fissure which becomes the cavity of the gland. The glands are secretory chiefly in the lower half of the ovary where the three glands join (fig. 12). Above the middle they become progressively flatter and narrower (figs. 13, 14), and, finally, above the placentas, they separate, becoming slender tubelike ducts (fig. 15) which open on the surface of the cone-shaped apex of the ovary (figs. 10, 11) in the blossom cup. These ducts were referred to as "intercarpellary fissures" by KERNS *et al.* (**15**) in their plate IV*A*.

During anthesis the nectar flows from the gland through the three ducts into the blossom cup. When nectar secretion is heavy, it may rise to the mouth of the corolla tube or may seep laterally between petals and sepals and collect on sepal or bract surfaces. The flow normally increases in volume till midday or early afternoon, after which it gradually subsides.

MATURE FRUIT.—Growth from blossoming inflorescence to mature fruit—about a twenty-fold increase in weight—results in various changes in structure and proportion of the floral parts (figs. 10, 11). There is no floral abscission, and, except for the withering of the style, stamens, and petals, the entire blossom develops into a fruitlet. The bract, sepal, and ovary tissues (including the glands)

FIGS. 4–17.—Fig. 4, floral diagram. Fig. 5, flower and subtending bract. ×1. Fig. 6, corolla and calyx. ×1. Fig. 7, petal with scales and opposite stamen. ×1. Fig. 8, cross section of ovary on level of placentas. ×2. Fig. 9, face view of placenta and part of carpel wall bearing it. ×2. Note stylar canal opening above placenta. Fig. 10, longitudinal section of flower and bract. Approx. ×1. Fig. 11, longitudinal section of mature fruitlet. ×1. Figs. 12–15, septal glands. ×10. Fig. 12, three glands, joined in center, at base of ovary. Fig. 13, glands below placental level. Fig. 14, glands at level of placentas. Fig. 15, three ducts at apex of the ovary. Figs. 16–17, diagrams of vascular system of flower. Fig. 16, longitudinal section. Fig. 17, vascular system split longitudinally and spread out in one plane. *BL C*, blossom cup; *BR*, subtending bract; *BR′*, bract of adjacent flower; *D*, dorsal carpellary bundle; *DU*, nectary duct; *GL*, septal gland; *LO*, locule; *O*, ovary limit; *P*, petal; *PL*, placenta; *S*, sepal; *ST*, episepalous stamen; *ST′*, epipetalous stamen; *STY*, style; *V*, ventral supply; *VB*, vascular bundle.

are prominent structures in the mature fruit.

The large, conspicuous bract is fleshy and widened at its base and bends over the flattened calyx surface, covering one-half of the fruitlet. The papery tip is dry and light brown.

The enlargement of the calyx is chiefly a result of continued growth by cellular division and enlargement in the synsepalous region. The calyx is now bent over horizontally, bringing the short free tips together into a peak that closes over a large chamber, the "blossom cup" (fig. 11). In shape, the blossom cup appears as though two cones were placed base to base with the taller one projecting outward under the sepals. Morphologically, it is composed of the apex of the ovary and the adaxial wall of the calyx, the two component parts being separated by a low circular ridge formed by the petal and stamen bases. The wall of the blossom cup normally becomes brown and horny, with the calyx lining usually lighter brown and less horny than the cup base. Within the blossom cup are the dried and shriveled remains of petals, stamens, and style.

The nectary ducts are conspicuous as brown and horny tubes extending down into the fleshy ovary from the base of the blossom cup. They are lenticular or lanceolate in cross section, with the tip resembling a fish tail or a broken end of a branch. The brown wall of the duct merges with the lining of the blossom cup.

Internally, the locules, which are proportionately large, distinct chambers at the flowering stage, become longer but relatively narrower and less conspicuous in well-developed fruits. This results from expansion of adjacent tissues, especially of the septa. The degree of this change varies widely with environmental conditions and with variety of pineapple.

Placentas bearing abortive ovules show some enlargement from the flowering stage but far less in proportion to that of the septal tissues. They increase about two to three times in size from the flowering stage to ripening, while the septal tissue enlarges about ten to fourteen times. Placentas bearing mature seeds, however, enlarge from four to ten times, depending on the number of fertilized ovules.

Vascular anatomy

For preliminary studies of the complex vascular system, thin radial slices of the inflorescence and fruit were cleared by simmering in 20% sucrose solution for a few hours, a method which proved more satisfactory, from the standpoint of rapidity and ease of handling, than clove oil, chloral hydrate, or lactophenol. Attempts were also made to trace the bundles by infusing aqueous nontoxic dyes through the peduncle or through localized incisions on floral surfaces. Among the dyes used, light green SF (12), fast green FCF, and vital red gave the best results. Since all these dyes except vital red are soluble in alcohol, only material prestained with vital red was found suitable for dehydrating and clearing by the usual alcohol-xylol-clove oil method.

For paraffin sections, material was fixed in formalin–acetic alcohol, then processed, nearly always, in normal-butyl alcohol with slight modifications of Zirkle's method. Sections were cut between 8 and 20 μ thick and were stained usually with safranin O and fast green FCF.

At the base of each flower, between the ovary and the inflorescence axis, is found, among other bundles, a ring of six strong

basic bundles (figs. 18, 19) which supply the carpels and the perianth above. Three of the six basic bundles, designated as *D–St–S* traces, give rise to the dorsal carpellary, outer stamen, and some of the sepal bundles. The remaining three alternate bundles, designated as *V–St′–P–S* traces, supply the ventral carpellary bundles, the three inner stamens, the petals, and, in part, the sepals.

The *D–St–S* traces run radially toward the base of the ovary, gradually swing to the outer margin of the locule, and proceed upward parallel with the locule wall (fig. 16). The dorsal carpellary bundle branches off below the level of the placenta and runs closely along the wall (figs. 16, 22). Near the top of the ovary it swings inward toward the base of the style, curving down slightly as it approaches the stylar canal, and ascends parallel with and directly outside of the stylar canal (figs. 25–29). The remaining *St–S* traces continue to the top of the flower and supply the outer stamen (*St*) and the sepal (*S*) (figs. 16, 26–29). There is one trace to each stamen. The exact number of traces to the sepal has not been determined, since other main bundles are supplied by the alternate basic bundles and the lateral supply from adjacent bract branches. The sepal, therefore, is supplied from three different sources.

The remaining three of the six basic bundles, *V–St′–P–S*, send out numerous branches toward the center of the ring at the base of the ovary to supply the carpel walls of the septal region. These are designated *V* bundles (fig. 20). The remaining parts of the *V–St′–P–S* traces, *St′–P–S*, curve outward and up to the top of the ovary where they give rise to the inner stamen (*St′*), petal (*P*), and sepal traces (*S*) (figs. 16, 25–29). Each petal receives three traces (fig. 26) which split into five, seven, and, finally, about twenty small bundles.

The *V* traces divide close to their point of departure from the *V–St′–P–S* supply, and split into two or more strands. Their branchlets show anastomosis as they supply the glandular regions, lying prominently alongside the gland fissures. Below the placentas, where the glands are developed to their maximum, these bundles lie in the folds of the convoluted secretory tissue (fig. 21); higher, where the gland is straight, they lie in single rows alongside the fissure (fig. 22). At the placental level these converge to the placentas where they ultimately supply the ovules, one trace to each ovule. A few of the bundles ascend in the ovarian septa higher than the placental lobe and then curve downward and laterally into the placenta. The traces in the placenta show further anastomosis without definite pattern.

The bract receives its main supply directly from the inflorescence axis, independently and free from the floral traces. One central bundle, *Br*, is characteristically different from the rest in that it makes an abrupt backward turn in its course prior to its escape from the axis within the radius of the flower it subtends (fig. 16). Many smaller bundles also enter the bract and split into about forty branches, of which more than half occupy the adaxial side (figs. 27–29).

Attempts to reconstruct the vascular structure of the entire inflorescence by tracing vascular connections between the florets were not successful because of the intricate network of small bundles in the inflorescence axis. It has been found, however, that out of thirty vascular connections observed between florets (adjacent, or skipping one or two florets), twenty-six were between florets on the steepest spiral of which there are

FIGS. 18–29.—Vascular supply of flower shown in successive transverse sections from below ovary to floral tube. Figs. 18–19, below ovary base. Figs. 20–21, ovary base. Fig. 22, just below placental region. Fig. 23, placental level. Figs. 24–25, top of ovary. Figs. 26–29, floral parts above ovary. (See legend to figs. 4–17 for meaning of letters.)

twenty-one on an inflorescence. Four florets showed connections between florets on one of the thirteen moderately steep spirals. From this study it appears as though the inflorescence may have been a spike or a panicle before these florets became united into a compact inflorescence-head during phylogenetic development. In general, the large mass of bundles is used up in centripetal order, the outermost bundles supplying the basal florets and the inner ones supplying the crown.

Histological changes during growth

Detailed observations were made to note changes in appearance, size, and structure of the vascular bundles; parenchyma of septum and noncarpellary tissue; epidermal lining of locule; gland epidermis and subepidermal layers; nectary ducts; blossom cup; and style. Studies were made from freehand and paraffin sections which were prepared for the study of vascular anatomy; these were supplemented by the celloidin method when necessary in sectioning hard and corneous parts of the mature fruit.

Vascular bundles.—The vascular bundles are collateral in relation to the xylem. In the young bud and flowering stages when the bundles are immature, the vessels are in various stages of development: they vary in diameter, in degree of lignification, and in extent of stretching of the spiral secondary thickening. The phloem parenchyma cells and some of the vessel cells contain protoplasts until about the flowering stage. The sieve tubes seem to increase in number during floral development, and the companion cells, although present, are not conspicuous. Granular materials and starch grains that are present in the xylem and phloem parenchyma cells become less in quantity after the flowering stage. Some bundles mature earlier than others, but all mature by the time the fruit becomes ripe. A zone of small parenchyma cells surrounding the vascular bundles in the noncarpellary tissue becomes modified into a sclerenchymatous bundle sheath. Such a sheath is lacking around the septal bundles.

Parenchyma of septum and noncarpellary tissues.—The ground parenchyma of the septum and noncarpellary tissues is composed of typical thin-walled parenchyma cells, more or less loosely arranged with small intercellular spaces evident throughout. The cells are small and chiefly oval in transection, with an ovoid or spindle-shaped nucleus in each cell. The wall is mainly cellulose. Cells with raphides are numerous and conspicuously larger than the other cells. Compound starch grains are a prominent cellular inclusion and are easily identified by their highly refractive hila which are rounded or slightly angular in the small grains and stellate in the larger. They become less conspicuous as the cells enlarge and disappear completely before the fruit ripens. Cell division is apparently complete before anthesis, and the increase in the volume of tissue is from cell enlargement. The walls of the fully distended cells appear much thinner. Later, when the cells begin to lose their turgidity very soon after ripening, the walls appear wavy in outline. In the overripe stage the walls, though essentially intact, show evidence of extreme softening and of some disintegration, appearing cloudy and less distinct in outline when stained with fast green. The softening of the walls takes place irregularly and appears more pronounced in the large cells containing

raphides. The disintegration seems to be more rapid in the noncarpellary tissue than in the septa. Various microörganisms may invade the overripe fruit and speed its disintegration.

LOCULE LINING.—The tissue lining the locule is, morphologically, of carpellary origin, but it merges completely with the noncarpellary tissue surrounding the ovary so that its limits cannot be determined. Differences in cell arrangement or cell type and size between the two regions—something structurally similar to that of the "core line" in apples (**16**)—are absent in the pineapple. It has been found, however, in field specimens with surface burn from copper compounds which has resulted in internal gummosis, that there is a narrow band of normal tissue lining the locule that apparently consists of ovary, stamen, and petal tissues which are not affected by surface injury.

The epidermis of the locule wall consists of small, compactly arranged cells which form a solid, continuous lining. They contain highly refractive bodies, probably of calcium oxalate. There is no evidence of secretion by these cells at any stage of growth. In the bud stage their outer walls already show some thickening, while the radial and inner walls are thin and delicate. In the postblossom stages when the internal tissues are enlarging, most of the epidermal cells become stretched laterally with the ovoid, oblong, or spindle-shaped nucleus lying against the inner or outer wall. During the ripening stage their outer walls are thick and heavy, but not cutinized, and the refractive oxalate crystals are prominent in many of the cells.

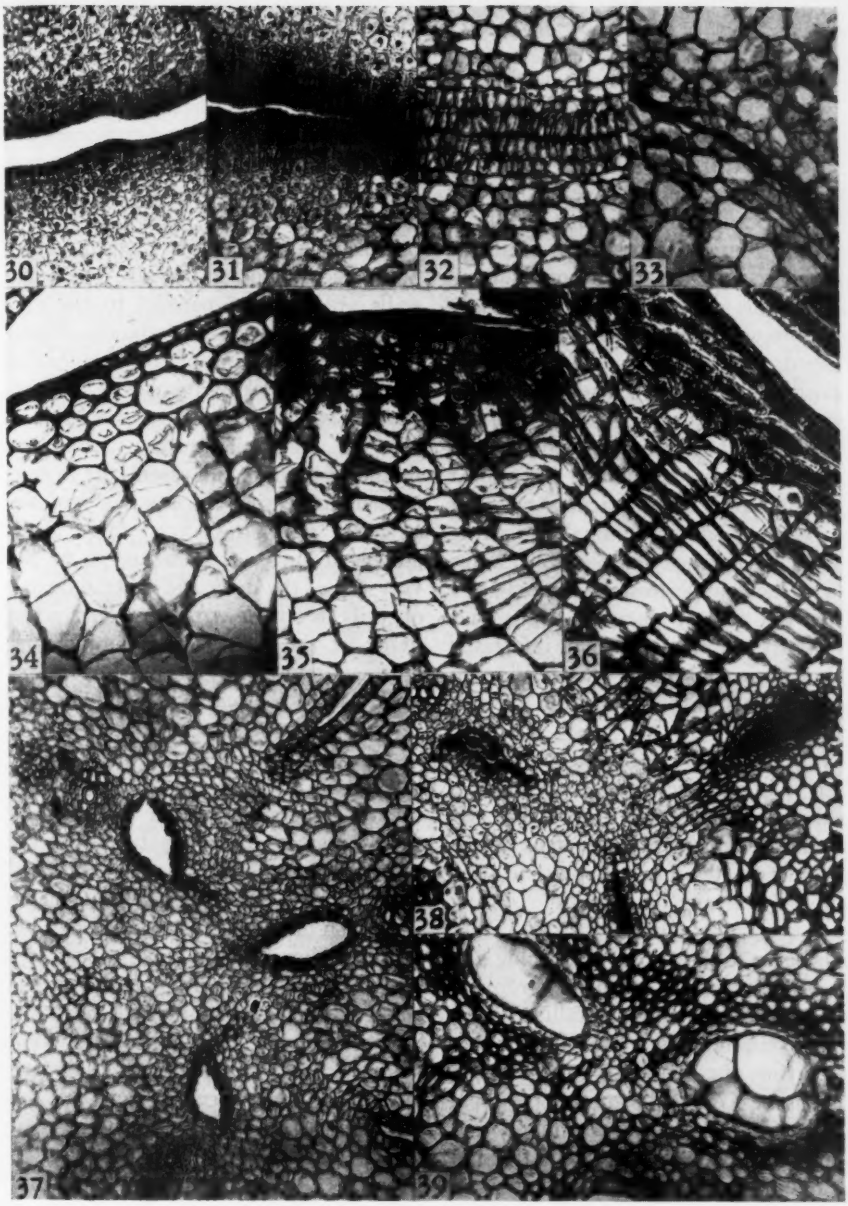
SEPTAL GLANDS.—The glandular region shows two distinct zones: (*a*) a uniseriate epidermal layer[2] and (*b*) a subepidermal zone[3] of three to five layers of cells (figs. 30, 31). The epidermal layer lining the cavities of the gland is similar to the epidermis of a secreting surface and is composed of cells which are more or less columnar in shape and densely cytoplasmic. In the bud stage they occasionally exhibit mitotic figures with an anticlinal plane of division. The nucleus, more or less centrally located, is large and spherical to oval, with one or two conspicuous nucleoli. The cell walls are not thickened, and there is no indication of cutinization or cuticularization.

The subepidermal layers exhibit a gradual transition in size and orientation from the epidermal cells to the unmodified parenchyma of the septum. Except for slight differences in shape, the cells immediately adjacent to the epidermis resemble the epidermal cells rather closely. Compound starch grains, which are abundant in the bud stage, are hydrolyzed to sugars during anthesis. These are carried outward to the epidermis and excreted into the cavity without any rupture of the wall or cell disintegration.

After active secretion is over, these cells very soon lose their granular appearance (fig. 32). The epidermis then looks like an ordinary epidermal layer, and the subepidermal cells merge with the septum parenchyma. The cells first show a meshwork of cytoplasm resulting from an increase in the number of vacuoles and finally exhibit a large central vacuole. The nucleus, obovate-oblong, and even elongate in extreme cases, appears constantly parietal in position. During fruit growth and enlargement,

[2] A similar layer was called "epithelial layer" by ACTON (1) in *Narcissus;* "palisade layer" by BUDNOWSKI (7) in Bromeliaceae.

[3] Called "epithem" by ACTON; "imbedding tissue" by BUDNOWSKI.



FIGS. 30–39.—Figs. 30–33, photomicrographs of glandular region at various stages. Fig. 30, young-bud stage. Fig. 31, flowering stage. Fig. 32, 3 weeks after blossoming. Fig. 33, ripe-fruit stage. Figs. 34–36, photomicrographs showing formation of periderm-like layer along wall of nectary duct. Figs. 34–35, 3 weeks after blossoming. Fig. 36, periderm-like layer in ripe-fruit stage. Figs. 37–39, photomicrographs showing closure of stylar canals. Fig. 37, cross section of style showing three open canals 1 day before flowering. Fig. 38, three canals completely plugged with mucilaginous substance 3 weeks after blossoming. Fig. 39, complete occlusion of canals by two to several tylosis-like cells, 1½ months after blossoming. All ×150. (Photographs by W. R. FISHER.)

these cells enlarge greatly in size but always remain smaller than the cells of the septum parenchyma. As a result of increase in size of the underlying cells, the two opposite epidermal layers bordering the gland cavity are brought together in intimate contact. In the ripe fruit the epidermis and the outermost subepidermal cells are stretched tangentially to a thin strip and in many flowers are crushed against similar layers on the opposite side (fig. 33). They then show necrotic reaction upon staining. On the other hand, there are some cells that do not undergo much stretching and appear in a single row, highly vacuolate, longer than wide, and distinctly smaller than the underlying parenchymatous cells. Some others show hypertrophy. Immediately below the nectary duct, the walls that are tightly compressed become infiltrated with suberin and lignin, thus sealing off the main part of the gland from the outside air.

NECTARY DUCTS.—The nectary duct, when it is first recognizable as such, is lined with a single layer of typical epidermal cells which are small, more or less isodiametric, densely cytoplasmic, and compactly arranged. Sometime during the young-bud stage the outer and inner walls begin to show some thickening, the cytoplasm becomes slightly shrunken, and aggregates of tiny crystalline bodies form within the cells. In the older buds—at the time the corolla tips protrude—a thin cuticle is detectible on the epidermal surface. Cuticularization is soon followed by lignification which proceeds through the middle lamella from the epidermal layer inward, usually affecting two or three cell layers.

About 3 weeks after blossoming, meristematic activity begins in the subepidermal cells in irregular patches, more frequently near the pointed ends of the lanceolate cavity. The behavior of the subepidermal cells (figs. 34, 35) shows close similarities to wound responses described by BLOCH (**5**) and SINNOTT and BLOCH (**22, 23**). These cells have all the distinctive features that vacuolated cells show when dividing to form young but vacuolated daughter cells. Their new walls are oriented parallel to the epidermal layer with the spindle-shaped daughter nuclei lying on each side. Added to this is the striking regularity in which the new walls are formed, one to three or four in tangential rows, within the mother-cell limits and essentially in continuous lines with the new walls of the adjacent cells (fig. 35). At a well-advanced stage, as in figure 36 at the very top of the duct, the suberized rows of cells resemble true periderm. The lining of the duct then is brown and horny, providing a solid, impervious wall. The epidermal and subepidermal cells are completely sclerified with a heavy infiltration of lignin and suberin, leaving no intercellular spaces between them. Cellular contents are largely tannin, mucilage, and crystals.

BLOSSOM CUP.—The base of the blossom cup and the nectary duct merge with each other, both showing the same process of lignification, suberization, and formation of periderm-like layer. The changes in the blossom cup, however, are somewhat more rapid and pronounced and involve more cell layers. Since the lining becomes rigid and incapable of stretching while the internal tissues are still enlarging, irregular stresses cause small cracks of various orientation to appear in the cup. Short and long vertical cracks are common on the ridge formed by the petal and episepalous stamens.

The upper half of the cup that is lined by the adaxial wall of the calyx

differs somewhat from the basal portion. The epidermal cells are small and flattened, compactly arranged, and contain small crystalline bodies. A thin cuticle is present. The hypodermal layer is composed of conspicuously large cells which become heavily lignified from the epidermis inward. The lignified, brown lining, however, is not always accompanied by the periderm-like layer. Cracking in these walls is confined to vertical splitting on the ridge between the petal and stamen bases.

STYLE.—The columnar three-lobed style is, morphologically, a result of fusion of three separate styles. In early ontogeny when the floral parts are first formed, the three styles are free or loosely united, with the adaxial sides deeply concave, and facing one another. During the process of growth and elongation, each leaflike part begins to fold inward ventrally, bringing the adaxial margins closely together and inclosing the stylar canal. The canal is, therefore, lined with epidermal cells of the adaxial surface that are roughly cubical and densely protoplasmic with a large central nucleus (fig. 37). They remain granular in appearance until anthesis, after which they begin to vacuolate. During anthesis a mucilaginous substance is secreted into the canal either from the stigma alone or from the stigma and the cells lining the canal. There is no cuticularization at any stage.

Cells on the abaxial surface are large and less granular, vacuolating rather early in the young-bud stage. During the bud stage the outer and inner walls begin to thicken, and tiny crystals appear in the cells. The outer wall continues to thicken in a pyramidal manner, begins to show a thin but definitely visible layer of cutin, and ultimately becomes cutinized. After anthesis, the walls become suberized and lignified. Parts of the abaxial surface show a periderm-like layer below the sclerified epidermis and merge with the lining of the nectary duct and blossom cup.

The stylar parenchyma consists of thin-walled cells which show a gradation in size from the small cells on the adaxial side to the largest on the abaxial side. Some contain starch grains. Those containing raphides are distinctly larger than the others. Withering and necrosis of the style normally take place progressively from the stigma to the base.

The stylar canals, which are open channels in bud and flowering stages, are completely closed in the mature fruit. There are at least two methods involved in the effective closure of the canal: first, by a mucilaginous plug; and, second, by cellular occlusion. The passageway is first obstructed by a secretion of mucilage or gum or by an accumulation of secretory products of the stigma and/or cells lining the stylar canal. This mucilaginous substance, more conspicuous in the upper half of the canal during and soon after anthesis, at subsequent stages is detected in the lower part of the style and appears more homogeneous, with a strong lignin reaction (fig. 38).

The second method of closure is initiated about a week or two after anthesis. This takes place in tissues near the base of the style. Probably in response to the presence of the mucilaginous substance higher in the style, the epidermal and subepidermal cells gradually enlarge and protrude into the cavity. In about 1½ months after flowering, these tylosis-like cells completely fill the canal at the base of the style (fig. 39). Observations have shown that these bulging tylosis-like structures, which extend into the cavity, are enlarged cells rather than pit membranes and, therefore, belong in the same category as the

tylosis-like cells (**9**) found in resin canals. Analogous structures are found in the internodal carinal canal of *Equisetum arvense* (**13**).

Summary

1. The structure of the inflorescence, the flower, and the fruit of the pineapple, *Ananas comosus* (L.) Merr., is described and interpreted.

2. The collective fruit is composed of numerous fruitlets and floral bracts which are connate with one another. Each hermaphroditic flower is trimerous in pattern and subtended by a prominent bract.

3. An important feature of the tricarpellate inferior ovary is the presence of septal glands which are, ontogenetically, the result of the incomplete fusion of adjacent carpel margins. The nectary duct, terminating each gland, provides a natural outlet from these glands into the blossom cup at the apex of the ovary. The glandular tissues show a definite transition from the secretory to the nonfunctional stage. The nectary duct and blossom-cup linings are transformed into an impervious, protective layer by combined processes of lignification, suberization, and formation of a periderm-like layer, thus protecting the fleshy tissues from the outside.

4. The stylar canals, which are open channels before anthesis, are closed by at least two methods: (*a*) mucilaginous plugging and (*b*) cellular occlusion by tylosis-like cells.

5. Two processes contribute to the increase in size of the fruit: cell multiplication in stages up to flowering and cell enlargement in the older bud to ripe-fruit stage. Cells of the internal tissues increase rapidly in size while the fruit is maturing, become fully distended upon ripening, and begin to show shrinkage when fully ripe. The overripe stages show softening and some disintegration of the walls and occasional cellular breakdown; dissolution, however, does not take place until microbial decomposition sets in.

6. Morphologically, the pineapple fruit is formed by the complete fusion of the fruitlets of an inflorescence and their subtending floral bracts to one another and to the inflorescence axis. This fusion has taken place during phylogenetic development. The flowers have typical inferior ovaries with the normal vascular supply for such ovaries of the appendicular type. Fusion of traces to the various organs has occurred whenever these bundles lie in the same radii, as is to be expected in an inferior ovary which is the result of maximum adnation (**8**). Fusion, likewise, has occurred between the lateral bundles of the subtending bract and some of those of the sepals. The bracts are intimately fused to their subtended flowers and to adjacent bracts. The bulk of the noncarpellary tissue between the ovaries is composed of sepal and bract tissues.

The writer wishes to express her appreciation to Dr. A. J. EAMES, under whose careful guidance this study was carried on, and to Dr. M. B. LINFORD for his suggestions and helpful criticisms. This work was done at Cornell University and the Pineapple Research Institute of Hawaii.

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LITERATURE CITED

1. ACTON, E. H. On the formation of sugars in the septal glands of *Narcissus*. Ann. Bot. **2**:53–63. 1888.
2. ANDERSON, C. E. Some studies in the floral anatomy of the Liliales. Thesis, Cornell Univ. 1940.
3. BAKER, J. G. Handbook of the Bromeliaceae. London. 1889.
4. BEER, J. G. Die Familie der Bromeliaceen. 1857.
5. BLOCH, R. Wound healing in higher plants. Bot. Rev. **7**:110–146. 1941.
6. BRONGNIART, AD. Mémoire sur les glandes nectarifères de l'ovaire dans diverses familles de plantes monocotylédones. Ann. Sci. Nat., sér. 4, **2**:5–23. 1855.
7. BUDNOWSKI, A. Die Septaldrüsen der Bromeliaceen. Bot. Arch. **1**:47–80, 101–105. 1922.
8. EAMES, A. J. The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. Amer. Jour. Bot. **18**:147–188. 1931.
9. EAMES, A. J., and MACDANIELS, L. H. An Introduction to Plant Anatomy. McGraw-Hill Book Co., New York. 1925.
10. GRASSMAN, P. Die Septaldrüsen. Ihre Verbreitung, Entstehung und Verrichtung. Flora **67**: 113–136. 1884.
11. HARMS, H. Bromeliaceae. *In* ENGLER and PRANTL's Die natürlichen Pflanzenfamilien **15a**:65–156. Section on *Ananas* 154–156. 1930.
12. HARVEY, R. B. Tracing the transpiration stream with dyes. Amer. Jour. Bot. **17**:657–661. 1930.
13. HOLDEN, H. S. On the occurrence of cavity parenchyma and tyloses in ferns. Jour. Linn. Soc. Bot. **47**:141–153. 1925.
14. HUME, H. H., and MILLER, H. K. Pineapple culture. II. Varieties. Fla. Agr. Expt. Sta. Bull. **70**. 1904.
15. KERNS, K. R.; COLLINS, J. L.; and KIM, H. Developmental studies of the pineapple *Ananas comosus* (L.) Merr. I. Origin and growth of leaves and inflorescence. New Phytol. **35**:305–317. 1936.
16. MACDANIELS, L. H. The morphology of the apple and other pome fruits. Cornell Univ. Agr. Expt. Sta. Memoir 230. 1940.
17. MEZ, CAROLO. Monographiae Phanerogamarum. IX. Bromeliaceae. Paris. 1896.
18. ———. Das Pflanzenreich. IV. Bromeliaceae. Section on *Ananas* 101–104. 1935.
19. MILES THOMAS, E. N., and HOLMES, L. E. The development and structure of the seedling and young plant of the pineapple (*Ananas sativus*). New Phytol. **29**:199–226. 1930.
20. SCHNIEWIND-THIES, J. Beiträge zur Kenntnis der Septalnectarien. Jena. 1897.
21. SIDERIS, C. P., and KRAUSS, B. H. Growth phenomena of pineapple fruits. Growth **2**:181–196. 1938.
22. SINNOTT, E. W., and BLOCH, R. Cytoplasmic behavior during division of vacuolate plant cells. Proc. Nat. Acad. Sci. (Wash.) **26**:223–227. 1940.
23. ——— and ———. Division in vacuolate plant cells. Amer. Jour. Bot. **28**:225–232. 1941.
24. SMITH, L. B. North American Flora **19**:61–228. (Xyridales) Bromeliaceae. 1938.
25. WITTMACK, L. Bromeliaceae. *In* ENGLER und PRANTL's Die natürlichen Pflanzenfamilien **2**: 32–59. 1888.

COMPARATIVE ANATOMY AND PHYLOGENY OF THE CASUARINACEAE

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Introduction

Most of the phylogenetic systems of the angiosperms in use today are based chiefly on the morphology of the flower; yet, there is a growing conviction among botanists that a natural classification of the plant kingdom must be founded on the combined evidence taken from all the fields of botanical endeavor. Thus, in the last three decades or so, there has been a serious attempt to use the available data derived from taxonomy, anatomy, cytology, paleobotany, and other fields of research to construct phylogenetic systems of classification. The role of wood anatomy in this effort to establish a natural system has been especially noteworthy (**28, 73, 74, 91, 110, 125, 129, 131**).

Because of the promise extended by the "anatomical method" in phylogeny, the present study was undertaken of the Casuarinaceae, a family, consisting of one genus (*Casuarina*), that has proved

to be one of the most perplexing groups of angiosperms to place in a natural system. TREUB (134) established a separate subdivision, the Chalazogamae, for it. It has been considered to be among the primitive groups of angiosperms by ENGLER and PRANTL (50), by RENDLE (115), and others; and it has occupied a key position as the most primitive of angiosperms in the phylogenetic systems of ENGLER and PRANTL (51), ENGLER and DIELS (49), JOHNSON (79), and WETTSTEIN (140). On the contrary, the family has been considered extremely reduced, yet always assigned a position tentatively, in the systems of HALLIER (66), BESSEY (22, 23), HUTCHINSON (69), and others. Indeed, *Casuarina* has been described as the "amphioxus of the plant kingdom."

The chief purpose of this anatomical investigation of the secondary xylem and, to a lesser extent, of the secondary phloem of the Casuarinaceae is to contribute toward a better understanding of the evolutionary status of the family. Another purpose is to provide a thorough anatomical description of the Casuarinaceae, necessary as evidence for the phylogenetic conclusions. It should be emphasized that the goal of this anatomical research is to provide a description of the genus, since the anatomical delineation of species requires much larger collections of specimens than were available for this investigation. A table listing the outstanding differences among species is presented. In a few cases anatomical data have seemed sufficient to warrant suggestions as to the taxonomy of the species. The external morphology and taxonomy of the family are described in standard systematic texts (49, 50, 79, 115).

Casuarina is distributed in Australia, in the islands of tropical and subtropical Polynesia, Melanesia, and northward to some of the islands of Micronesia and to the Philippines, and westward to the Malayan Peninsula, India, and the Mascarene Islands in the Indian Ocean. The majority of the species have fairly limited ranges and are restricted pretty largely to Australia and the adjacent islands of Tasmania and New Caledonia. Several species, especially *C. equisetifolia*, have been introduced in some of the southern states of the United States, chiefly Florida, Arizona, and California, for ornamental purposes and, owing to their ability to grow rapidly in alkaline or brackish soils, for purposes of dune reclamation along the seacoasts.

The *Index Kewensis* (71) lists sixty valid species of *Casuarina*, but it seems necessary to modify this number somewhat. Four binomials are listed as synonyms in the *Index Kewensis* which should probably be considered valid species: *C. baxteriana* Miq. (89), *C. humilis* Otto & Dietr. (44, 108), *C. muelleriana* Miq. (89), and *C. paludosa* Sieb. (89). These four species would raise the number to sixty-four, but five binomials listed as valid species in the *Index Kewensis* are here, on the basis of the available literature, considered as synonyms: *C. horrida* Herb. (63), *C. leptotrema* S. Moore (63), *C. rumphiana* Miq. (126), *C. spinosissima* Gardn. (63), and *C. brunoniana* Miq. (93). Five additional binomials which will be referred to in this investigation have been reduced to synonymy (71). *C. muricata* Roxb. is a synonym of *C. equisetifolia* Forst. (45), *C. quadrivalvis* Labill. a synonym of *C. stricta* Ait. (20), *C. leptoclada* Miq. a synonym of *C. suberosa* Otto & Dietr. (20), *C. tenuissima* Sieb. a synonym of *C. torulosa* Ait. (20), and *C. lehmanniana* Miq. a synonym of *C. humilis* Otto & Dietr. (20).

Several authors have described the anatomy of certain species of *Casuarina*,

and these authors should be briefly acknowledged. BOODLE and WORSDELL (25) described the structure of the young stems and several specimens of the older wood of several species. They concluded that *Casuarina* demonstrates anatomical characters different from most of the other groups of angiosperms; but they stated that similarities of the anatomy, such as ray and parenchyma structure, with that of *Quercus* and *Corylus* were obvious. Although SOLEREDER's (125) description of the family is largely based on young stems and only to a lesser degree on older wood, there is no disagreement between his description and the writer's observations.

GÖPPERT (65) presented a very general description of young stems and the chief cells of older wood. He was, apparently, the first to notice the broad bands of metatracheal parenchyma in *Casuarina*, but he considered the bands as concentric rays. POISSON (108) summarized previous anatomical work of the genus and then presented a taxonomic monograph. LECOMTE (86) published an article in which he described the young stems of several species and accounted for many of the chief features of older wood. PRAËL (109) described in a general way the young and old stems of four species (*C. equisetifolia*, *C. quadrivalvis*, *C. muricata*, and *C. nodiflora*). An interesting series of papers was published by MORINI. Two of them were concerned with the anatomy of young stems as well as several features of the secondary xylem of older stems (95, 96). HOULBERT (68) described accurately the rays, the parenchyma, and the frequency and size of vessels in the older wood of five species. BARGAGLI-PETRUCCI (14) described the older wood of *C. sumatrana*. MOLL and JANSSONIUS (94) described the macroscopic and microscopic details of the structure of the old wood of the two species of *Casuarina* found in Java: *C. equisetifolia* Forst., and *C. montana* Miq. var. *tenuior* Leschen. TIPPO (129) gave a brief account of the family Casuarinaceae based on a study of eleven species. In general, the author's investigation bears out the findings of TIPPO but differs in a few details.

Over a long period of years, taxonomists and systematists have established that there are apparently certain trends in the evolution of the external morphology of angiosperms, especially of the flower. Similarly, during the last 30–40 years, plant anatomists have worked out the lines of phylogenetic specialization of the stele, particularly of the secondary xylem. These trends of phylogenetic specialization may be stated in the form of dicta (129, 131):

1. The protostele is primitive, and the siphonostele and dictyostele are derived (72, 74).

2. In angiosperms the woody stem of trees and shrubs is primitive, while the herbaceous stem is derived (12, 46, 76, 77, 124).

3. Among the imperforate tracheary elements, evolution has advanced from tracheids, to fiber-tracheids, to libriform wood fibers (9, 13, 74).

4. There is a decrease in length of the imperforate elements as they become more specialized (6, 8, 13).

The length of the cambial initials decreases as specialization proceeds in the secondary xylem of gymnosperms and dicotyledons (6). Although the percentage of elongation of the fibers in differentiation increases as the cambial initials decrease, the amount of decrease in the cambial initials is so much greater than the proportional increase in fiber length during ontogenetical differentiation that the generalization that fibrous

elements decrease with specialization still holds (35).

5. In the angiosperms evolution has proceeded from scalariform tracheids to circular bordered pitted tracheids (74).

6. The septation of fiber-tracheids and libriform wood fibers is a specialized character (129).

7. The solitary pore arrangement of vessels is less advanced than the aggregate groupings, such as pore multiples, pore clusters, and pore chains (FROST, unpublished data).

8. The diffuse-porous condition is primitive, and the ring-porous is specialized (56, 64).

9. Vessels with thin walls are more primitive than those with thick walls (56).

10. Vessel elements which are long, small in diameter, and angular in transverse section preceded those which are short, large, and circular in transverse outline (6, 13, 56).

As the secondary xylem of gymnosperms and angiosperms becomes more specialized, the cambial initials decrease in length (6). The vessel elements, in most cases, are equal to the cambial initials in length (6, 30); hence, the vessel elements also tend to decrease in length.

11. Vessel elements with scalariform perforation plates appeared in plants before vessel members with a single (simple) opening (10, 13, 56, 57, 74).

12. Among the vessel members with scalariform perforation plates, the type with numerous bars and narrow perforations is primitive and the type with few bars and wide openings is advanced (57).

13. Vessel elements with scalariform perforation plates may have openings with complete borders, with borders to the middle of the perforation, with borders only at the end of the openings, or with nonbordered apertures. Specialization has proceeded in the order named (57).

14. Vessel elements with long, sloping end walls are less advanced than those with transverse end walls (10, 13, 56, 57).

15. The evolution of the intervascular pitting on the side walls of the vessels has been from scalariform through transitional to opposite and finally to alternate (13, 56, 58).

16. Spiral thickenings in vessels are indicative of specialization (58).

17. Heterogeneous rays (those with both vertically and radially elongated cells) are less specialized than homogeneous rays (those in which all the cells are radially elongated) (15, 16, 17, 83).

KRIBS (83) has shown that the phylogenetic order is from the primitive heterogeneous type I ray, through the transitional heterogeneous types IIA and IIB, to the homogeneous type I, and finally to the highly specialized homogeneous type II rays.

18. The diffuse parenchyma arrangement is primitive, and the metatracheal and various paratracheal types, such as vasicentric, are more specialized (84).

19. Woods with storied structure are more advanced than woods with nonstratified cells (7).

Most of these phylogenetic sequences have been worked out by methods which are founded on the work of BAILEY and TUPPER (13). These authors made an extensive study of cell size in hundreds of plants drawn from all the major groups of the vascular plants; and they discovered that the tracheary elements in the vascular cryptogams are very long, in the gymnosperms intermediate in length, and in the angiosperms relatively short. They also discovered that the specialization of the vessel element is highly correlated with the reduction in length of the tracheary cells. TURRILL (135) and

TIPPO (131) pointed out that the direction of the lines of specialization listed previously have been established either by founding them on paleobotanical, comparative morphological, and developmental studies of all the major vascular plant groups, or, using the work of BAILEY and TUPPER as a starting-point, by the methods of association, correlation, exceptions, and sequences listed by FROST (56). These phylogenetic trends, therefore, have been formulated independently of any preconceived theories of angiosperm classification; and any suggestion made by the anatomist may be accepted as evidence obtained by independent methods and thus relatively free of bias.

The phylogenetic lines, once they have been revealed, may be used to interpret data acquired from anatomical study of a group of plants. To determine the phylogeny of a group of plants, an attempt is made to harmonize the evolutionary status of the group indicated by the use of the anatomical trends of evolution with the relationship of the group deduced from data obtained from other fields of botany, such as floral anatomy, paleobotany, cytology, nodal anatomy, embryogeny, pollen morphology, etc.

The secondary phloem of the angiosperms has not been studied so thoroughly as the wood, and any dicta pertaining to the lines of specialization are difficult to state. HEMENWAY (67) classified the sieve-tube elements into three types, although intergradations are common. In the first type, the elements are long, of small diameter, and have tapering ends. The end walls are very oblique, extending from one-half to one-fourth the length of the cell. The sieve areas on the side walls are identical with those of the end walls, and the areas are all of the same degree of specialization. The elements of this type do not form chains or sieve tubes and must therefore be given the term of sieve cells rather than sieve-tube elements (36).

The second and third types form continuous tubes, or sieve tubes, and the cells composing the tubes, therefore, are sieve-tube elements (36). In the second type (67), the elements are shorter, broader, and have less oblique end walls with two to ten sieve plates on each. The lateral sieve areas (36) are poorly developed, being vestigial and functionless, while the functional sieve areas are localized and specialized to form compound sieve plates on the end walls. In the third type, the sieve-tube elements are relatively short and broad and have nearly transverse end walls, with usually a single sieve plate (67) or simple sieve plate (36). The sieve plates or lattices (67) on the side walls are poorly developed, vestigial, and functionless, or absent entirely.

In the monocotyledons the sieve cell is primitive and the sieve-tube element is advanced (36). Evolution proceeded to sieve-tube elements that had oblique end walls with compound sieve plates and less conspicuous sieve areas on the side walls to sieve-tube elements that had nearly transverse to transverse end walls with a simple sieve plate, and inconspicuous sieve areas on the side walls. Although there is little doubt that sieve-tube elements in dicotyledons have developed as described above (53, 67, 88), there is no reason to assume that the phloem and xylem have evolved concomitantly in all groups of dicotyledons.

Material and methods

There follows below a list of the twenty-nine investigated species. The determination of the validity of species names was based chiefly on the list of

valid names and synonyms in the *Index Kewensis*. Where a perusal of the literature indicated that synonymy exists, the synonyms are placed in parentheses. After each species name are listed the specimens that were available. The letter *Y* indicates that the specimens were obtained from the wood collection of the School of Forestry, Yale University; an *H* indicates the source as the Biological Laboratories of Harvard University; a *D*, the Council for Scientific and Industrial Research, Melbourne, Australia; a *C*, the Imperial Forestry Institute, Oxford, England; and an *R*, the Forestry Products Research Laboratory, Aylesbury, Bucks, England. In each case, the number is that assigned to the specimen by the respective institution. Several specimens, sent to the University of Illinois from various other sources, are indicated by the letter *I*. Whether the specimens are young (near the pith) or old wood (at some distance from the pith) is also noted.

C. stricta Ait.: old wood—H21883, H21730, H21917; young wood—H3393, H15282, I-15, I-16. (*C. quadrivalvis* Labill.): old wood—H20244, H21913, Y19328, R2285; young wood—H15272, H15267.

C. glauca Sieb.: old wood—Y32245, Y32788, D1708, D1711, D7526, C12645, R2861, R2284; young wood—H967, H3393, H3396.

C. equisetifolia Forst.: old wood—H3402, H7627, H23307, Y23437, Y27756, Y34941, D4212, D4213, D7527, C4516; young wood—H295, H3407, H3408, H4143, H4144, H4330, H15273, H25188, I-20, I-23. (*C. muricata* Herb.): old wood—C3865; young wood—H9941, H15276.

C. suberosa Otto and Dietr.: old wood—Y9698, Y32732, Y32733, D1530, D3278, D3279, D3280, D3281, D3282, D7528, I-27; young wood—H3416, H4142, H15268, H15275, H15278, H15280, H15288, H15289. (*C. leptoclada* Miq.): old wood—D1652, D1653.

C. cunninghamiana Miq.: old wood—H20868, Y11102, D3128, D3151, D5152, D3258, D7529, D3263, R1686, R2559, C4009; young wood—H14140, H15277, H15279, I-25.

C. distyla Vent., sen. str. Macklin: old wood—Y19329, Y16369; young wood—H3394, H3395, H15285.

C. fraseriana Miq.: old wood—H4139, Y19668, Y38020, D3136, I-13, I-30; young wood—H5164, H4138, H15284.

C. torulosa Ait.: old wood—H3404, H7628, H15286, H19055 (same as Y32468), Y32683, Y32686, D1531, D1603, D1606, D3137, D7531, I-28; young wood—H3391, H3398, H3403, H3405, H5163, H15281. (*C. tenuissima* Sieb.): young wood—H9943, H15283.

C. decaisneana F. v. Muell.: old wood—D6383.

C. cambagei R. T. Baker: old wood—D6335, D6336, C12644.

C. collina Poiss.: old wood—Y14140, Y14183.

C. deplancheana Miq.: old wood—Y14240.

C. dialsiana (authority unknown): old wood—I-32.

C. junghuhniana Miq.: old wood—Y22133.

C. lepidophloia F. v. Muell.: old wood—I-31.

C. montana Leschen.: young wood—H5165.

C. montana Leschen. var. *tenuior* Miq.: old wood—Y31836, Y31837.

C. nodiflora Forst.: old wood—H20869, Y28390, C2907. (*C. rumphiana* Miq.): young wood—C1734.

C. poissoniana Schlecht.: old wood—Y14663.

C. sumatrana Jungh.: old wood—H23437, Y22134, C6665.

C. nana Sieb.: old wood—H15271; young wood—H15271.

C. humilis Otto and Dietr. (*C. lehmanniana* Miq.): old wood—Y16368; young wood—H15270.

C. inophloia F. v. Muell. and Bailey: old wood—Y13055, D1088, D7350; young wood—H15269, R7310.

C. luehmanni R. T. Baker: old wood—Y13064, D2285, C12646.

C. bicuspidata Benth.: old wood—D6688.

C. acutivalvis F. v. Muell.: old wood—D6693.

C. baxteriana Miq.: old wood—D6694.

C. muelleriana Miq.: old wood—D6695; young wood—D6409.

C. corniculata F. v. Muell. (*C. horrida* Herb.): young wood—D6689.

C. paludosa Sieb.: young wood—H15274.

C. spp. (species undetermined): old wood—H21720, H21740, D3155; young wood—H3400, H3410, H3413, H9682, H15287, H24687, H24688, H24689.

A total of 170 specimens was examined. Of these, 104 were old wood and 66 were young wood. With the exception of *C. corniculata* and *C. paludosa*, old-wood specimens of all the species were examined.

Ten slides were made of each specimen, two unstained and eight stained. The technique followed was that outlined by BAILEY (**3**), JEFFREY (**75**), and WETMORE (**139**). All old-wood specimens were cut into small blocks, boiled, cooled, and then softened in hydrofluoric acid (48 or 52%). All old-wood specimens were washed and dehydrated and, at first, were then stored in glycerin-alcohol until the time of sectioning. When it was found that the wood of *Casuarina* was difficult to section, the specimens after dehydration were impregnated and imbedded in celloidin (Parlodion) and then stored in glycerin-alcohol. About one-half the old-wood specimens were treated thusly before sectioning. All the young-wood specimens were imbedded in celloidin.

Transverse, radial, and tangential sections were cut on a Reichert sliding microtome at 13–15 μ for all specimens listed except five, for which slides had been previously prepared at Harvard University. Because many of the cross sections were badly shattered, very often additional transverse sections were cut at 20–22 μ. All sections were stained by Heidenhain's iron-alum hematoxylin method—or similarly using a methyl cellosolve hematoxylin method (**78**)—and counterstained in safranin O. A few sections of young wood with bark attached were stained lightly with only hematoxylin to facilitate study of the phloem.

Macerations with JEFFREY's maceration fluid (**32**) were made of one specimen of each species except three: *C. poissoniana*, of which none was successful, and *C. paludosa* and *C. corniculata*, of which only young-wood specimens were available. Four additional specimens of *C. suberosa* were macerated in order to obtain information on the variation in length of the elements among several specimens of a single species.

The terms used in the anatomical descriptions comply with those approved by the Committee on Nomenclature of the International Association of Wood Anatomists (**37**) and as clarified and illustrated by later authors (**26, 111**). The diagnostic characters used are those listed by TIPPO (**130**), but some reference has been made to previous lists (**112, 113**).

The imperforate tracheary elements are classified as tracheids if the bordered pit-pairs are equal to or larger than the corresponding pit-pairs on the side walls of the vessel elements, and are fiber-tracheids if the pit-pairs are conspicuously smaller than the corresponding pit-pairs on the vessel members (**9**). Gelatinous fibers (**116**), in the case of the Casuarinaceae, are fiber-tracheids in which the inner wall layers have become gelatinized (unlignified and probably of hemicellulose). In describing the relative thickness of the walls of the fibrous elements, CHATTAWAY's (**34**) classification has been used:

With reference to the radial wall:
- Very thin—lumen much greater than the thickness of walls.
- Thin—lumen greater than thickness of walls.
- Thick—lumen much less than thickness of walls.
- Very thick—lumen almost completely closed.

The classification used for length of fiber and vessel elements is that proposed by the Committee on the Standardization of Terms of Cell Size of the Interna-

tional Association of Wood Anatomists (38):

	Fibers (μ)	Vessel elements (μ)
Extremely short	Less than 500	Less than 175
Very short	500– 700	175– 250
Moderately short	700– 900	250– 350
Medium-sized	900–1600	350– 800
Moderately long	1600–2200	800–1100
Very long	2200–3000	1100–1900
Extremely long	Over 3000	Over 1900

The measurements of the vessel members are the total lengths—that is, from the tip of one "tail" to the tip of the other "tail"—as recommended by CHALK and CHATTAWAY (30, 31). All measurements of fiber and vessel elements were made from macerated material. A statistical analysis has been made of these measurements as suggested by DESCH (42) and by RENDLE and CLARKE (117, 118). Measurements of the elements were always from old wood, complying as far as possible with the dictum that such studies should be made from comparable parts of the tree (27, 43). The measurements, as such, were taken to provide a general idea of the relative length of the fiber and vessel elements of the genus rather than to give information on the differences among the species.

Fifteen to twenty counts were made of the number of vessels per square millimeter of 100 old-wood specimens and 53 young-wood specimens, chiefly to yield data on the range of variation. The time involved in any additional counts would have been out of proportion to the value derived. The number classes used for the number of vessels per square millimeter are those of CHATTAWAY (34):

Up to 2	Very few
2–5	Few
5–10	Moderately few
10–20	Moderately numerous
20–40	Numerous
Over 40	Very numerous

A classification for the thickness of the wall has not been discovered in the literature. Since certain species have vessels whose wall thickness rarely exceeds 6 μ and other species have a majority of vessels with greater thickness, the dividing line between thin and thick was arbitrarily set at 6 μ.

The vessel diameters were measured for a hundred vessels on transverse sections of 94 old-wood specimens and 49 young-wood specimens. The size classes employed are those of CHALK (29):

Extremely small	Up to 25 μ
Very small	25– 50
Moderately small	50–100
Medium-sized	100–200
Moderately large	200–300
Very large	300–400
Extremely large	Over 400

FROST (57) has classified scalariform perforation plates as to the number of bars: plates with five or fewer bars are placed in the few class, those with five to fifteen bars are placed in the intermediate class, and those with fifteen or more in the many class. Furthermore, scalariform perforation plates with perforations (measured from bar to bar) of 6 μ or less are classified as narrow, while those with openings of more than 6 μ are classed as wide.

The size classes for the intervascular and ray-vessel pit-pairs have been delimited by RECORD and CHATTAWAY (113). The classes for the intervascular pit-pairs are: minute (up to 4 μ diameter); small (4–7 μ); medium-sized (7–10 μ); large (10–15 μ); very large (over 15 μ). The size classes for the ray-vessel pit-pairs are: five (not more than 7 μ in diameter); medium (7–10 μ); coarse (more than 10 μ).

The classification of rays used is that of KRIBS (83). The width and height of rays have little phylogenetic significance,

and they vary considerably among specimens of the same wood, so that no classification has been accepted other than to suggest arbitrary groupings. RECORD and CHATTAWAY (113) have proposed these groups for describing ray width in tangential aspect; one to two cells, three to four cells, five to ten cells, ten or more cells. The following groupings of ray heights have been proposed by the same authors; fewer than 25 cells high, 25 to 50 cells, 50 to 100 cells, and over 100 cells. These groupings have been followed somewhat in the description of the genus.

The terms used for describing distribution of xylem parenchyma are taken in the main from BAILEY and HOWARD (11). The types are:

Apotracheal	Paratracheal
diffuse	scanty
metatracheal	abundant
terminal	vasicentric
initial	aliform
	confluent
	banded

Definitions for these types occur in the report of the Committee on Nomenclature of the International Association of Wood Anatomists (37), but they have been clarified by KRIBS (84) and by BAILEY and HOWARD (11). In describing the metatracheal parenchyma, the following classification proposed by RECORD and CHATTAWAY (113) is employed: uniseriate or narrow bands (typically fewer than three cells wide); coarse bands (four or more cells wide).

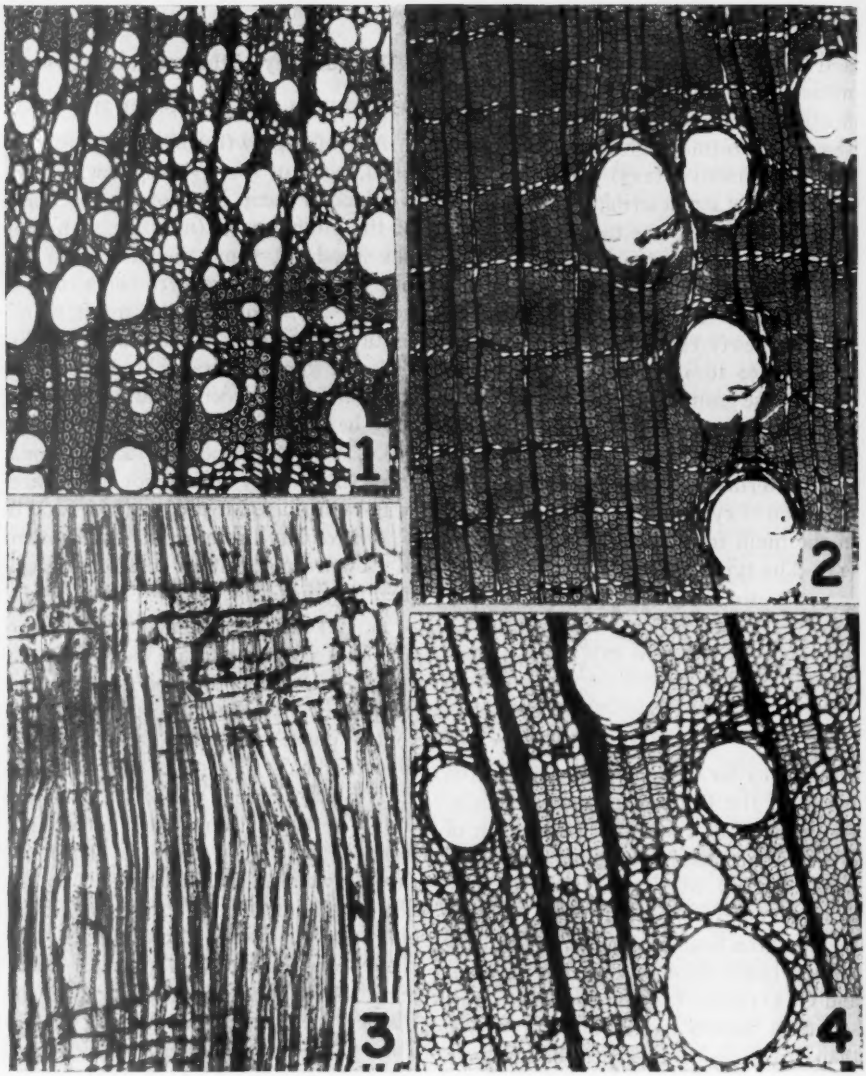
One stained slide of each specimen prepared has been forwarded to the Biological Laboratories of Harvard University, and one set has been retained by the Department of Botany at the University of Illinois. One unstained and one stained slide of each specimen have been added to the wood collection of the School of Forestry at Yale University.

The anatomy of the secondary xylem

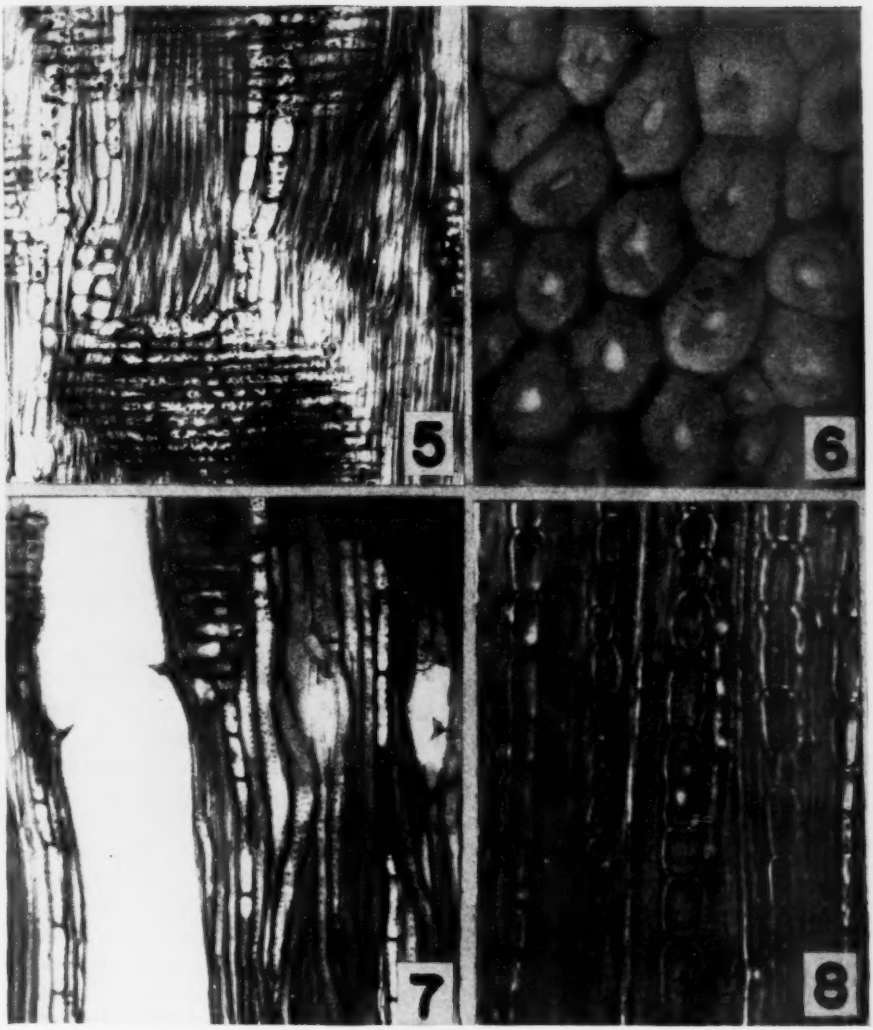
ANATOMICAL DESCRIPTION OF THE GENUS

Although growth rings are poorly defined, none of the species shows an entire lack of them. Most of the specimens of the young wood (near the pith) and old wood (at some distance from the pith) demonstrate growth rings (figs. 1, 10, 26, 27), probably the result of climatic fluctuations. *C. stricta*, generally, does not show any traces of growth rings in young or old wood, although rings may be noted in certain specimens (fig. 26). *C. distyla*, *C. nodiflora*, and *C. sumatrana* (fig. 2) may or may not show traces of growth rings. Most of the old wood of *C. equisetifolia* has a slight development of growth rings, but an occasional specimen may not.

IMPERFORATE ELEMENTS.—Of the various types of imperforate elements, only tracheids and fiber-tracheids are present. Thin-walled tracheids occur in a vasicentric position and the thickest-walled fiber-tracheids in regions farthest from the vessels. There is a gradual transition from the large, thin-walled, often malformed (fig. 7), vasicentric tracheids through thicker-walled tracheids to the fiber-tracheids (figs. 1, 4, 11, 39). In the old wood of twenty-three species, tracheids predominate (fig. 3), the relative numbers varying from 20 to 95% of the tracheary elements, but ranging mostly from 70 to 95%. Fiber-tracheids predominate in the old wood of *C. equisetifolia* (80–95%) (figs. 4, 5), *C. suberosa* (10–90%), and *C. humilis* (70%). Only tracheids occur in the wood of *C. dialsiana*. The wood of young twigs possesses, in general, a higher proportion of thin-walled tracheids than the old wood of the same species. The walls of the imperforate tracheary elements in the old wood of nearly all the species must be



FIGS. 1–4.—Fig. 1, *C. suberosa:* transverse section to show indefinite growth rings; tendency toward ring porosity; solitary distribution of vessels; and vasicentric, thin-walled tracheids with gradual transition to thicker-walled fiber-tracheids at some distance from vessels. ×85. Fig. 2, *C. sumatrana:* transverse section to show absence of growth rings; moderately few, medium-sized to moderately large vessels; diffuse porosity; solitary distribution of vessels; narrow metatracheal parenchyma; and nearly complete absence of diffuse parenchyma. Notice also thick-walled fibers, which in this species, according to definition, are fiber-tracheids, or even tracheids with pit-pairs equal in diameter to corresponding pit-pairs on vessel elements. ×75. Fig. 3, *C. fraseriana:* radial section to show wood in which fibrous elements are nearly all tracheids. Notice heterogeneous rays. ×100. Fig. 4, *C. equisetifolia:* transverse section demonstrating wood in which fibrous elements are mostly thick-walled fiber-tracheids. Notice lack of aggregate rays; narrow metatracheal and rare diffuse parenchyma; very small to medium-sized, circular and solitary vessels; vasicentric, thin-walled tracheids; and thick-walled fiber-tracheids at some distance from thin-walled vessels. ×100.



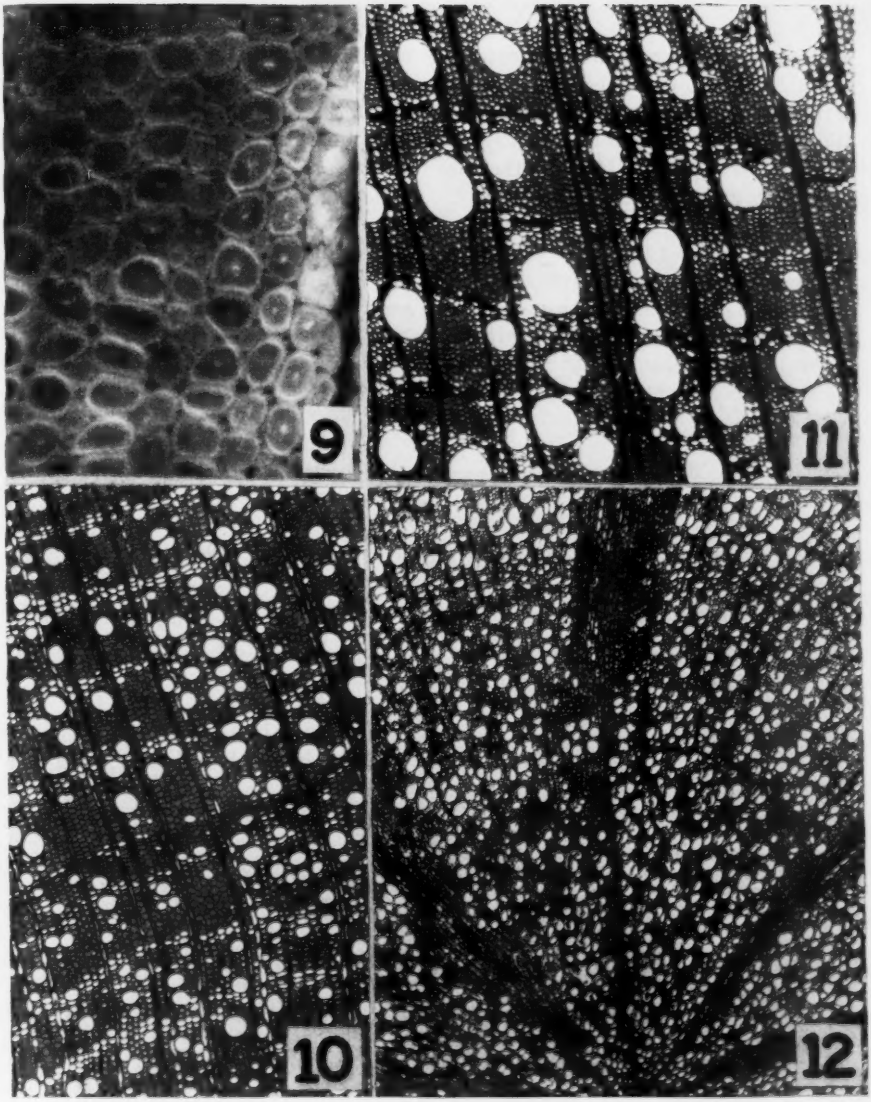
FIGS. 5–8.—Fig. 5, *C. equisetifolia:* radial section to show wood in which fibrous elements are mostly fiber-tracheids. Notice heterogeneous rays, chambered parenchyma, and appearance of parenchyma which, in transverse aspect, is distributed as narrow metatracheal parenchyma. ×100. Fig. 6, *C. equisetifolia:* transverse section to illustrate very thick-walled fiber-tracheids. Notice reduced bordered pit-pairs. ×1000. Fig. 7, *C. equisetifolia:* radial section to demonstrate short, somewhat malformed vasicentric tracheids and oblique end walls on vessel elements. ×100. Fig. 8, *C. cambagei:* radial section illustrating gum deposits in fibrous elements. Gum often gives impression that fibers are septated. ×830.

classified as thin to very thick. In the majority of the species, the walls of the fiber-tracheids become so thick as nearly to occlude the lumen (figs. 2, 6, 13, 20, 24). In the wood of young stems, in most cases, the wall varies from thin (occasionally very thin) to very thick, although there is a higher proportion of thin-walled elements correlated with the higher percentage of tracheids. Even though the walls of the tracheary elements are frequently very thick, the thick-walled elements must be called fiber-tracheids, as they possess circular bordered pit-pairs (fig. 6). No libriform wood fibers have been seen. The bordered pit-pairs in the walls of the imperforate elements are circular to oval (fig. 19), or rarely elongate, and range from 1.5 to 6.0 μ. The most frequent range in the old wood is from 2.5 to 4.5 μ, and in the young wood from 2 to 4 μ. These pit measurements seem small for wood which is largely composed of tracheids, but this seeming discrepancy is accounted for when it is noted that the intervascular pitting of the vessels is minute to small and only occasionally medium in size (fig. 21). The smaller pit-pairs of the fiber-tracheids have elongated and crossed inner apertures. No septate fiber-tracheids are present; but "gum" deposits in some of the fiber-tracheids, noted especially in *C. cambagei* (fig. 8), often give a false impression of septation. Gelatinous fibers are modified fiber-tracheids and are found occasionally scattered in the old wood, but they are especially common in young wood (fig. 9), where they occur in solid blocks or in irregular areas as seen in transverse section.

Measurements of fiber length were made of five specimens of *C. suberosa* and of one specimen of each of twenty-five other species. No measurements were made of the fibers of *C. poissoniana*, *C. corniculata*, and *C. paludosa*. The most frequent ranges of fiber lengths in microns for the five specimens of *C. suberosa* are 615–1086, 638–929, 671–1007, 783–1150, and 895–1176. The means in microns for one hundred measurements in each of the same five specimens are 835, 805, 814, 918, and 963. The variation in this one species indicates that the other data, computed from measurements of a hundred fibers from only one specimen of each of twenty-five species, should be considered for the genus as a whole. The range of fiber lengths for the three thousand measurements on thirty specimens of twenty-six species is from 224 (extremely short) to 2016 μ (moderately long). The most frequent range is 649 (very short) to 985 μ (medium-sized), and the mean for the genus is 799 $\pm$ 5.6 μ (standard deviation 306.8 $\pm$ 3.9) (moderately short).

Spiral thickenings are found in all or nearly all the imperforate tracheary elements of six species and are absent in fifteen species. In four species, certain elements possess spiral thickenings and others do not. The other four species may be classed as variable because in only an occasional specimen are the thickenings present. *C. suberosa* is very variable in this respect.

VESSELS.—The number of vessels per square millimeter of the old wood in transverse aspect is exceedingly variable, both among the species and among the various specimens of a species (table 1). In the genus as a whole, the range of species means shows, on the basis of a hundred specimens, a variation from moderately few (4 spp.) (fig. 2), to moderately numerous (5 spp.) (fig. 11), to numerous (5 spp.), to very numerous (13 spp.) (figs 10, 28, 32). *C. poissoniana* with a species mean of 8 and *C. bicuspidata*



FIGS. 9–12.—Fig. 9, *C. distyla:* transverse section of young stem in which many of thick-walled fiber-tracheids have become transformed into gelatinous fibers. ×500. Fig. 10, *C. muelleriana:* transverse section to show very numerous, extremely small to very small vessels in old wood. Notice also indefinite growth rings, slight tendency toward ring porosity, the uniseriate and multiseriate rays, and narrow metatracheal and diffuse parenchyma. ×75. Fig. 11, *C. glauca:* transverse section of old wood to demonstrate moderately numerous, very small to moderately small, circular vessels. Notice also transition from thin-walled vasicentric tracheids to thick-walled fiber-tracheids farther from vessels, the narrow metatracheal and scarce diffuse parenchyma. ×75. Fig. 12, *C. montana:* transverse section of young stem to show very numerous and extremely small to moderately small vessels of wood near pith. Notice widening of uniseriate rays to form multiseriate rays and aggregation of both types to form small aggregate rays. Parenchyma is diffuse and narrow metatracheal; vessels are solitary. ×37.

with 304 vessels per square millimeter illustrate the two extremes. The average of means for the genus is 66.7 per square millimeter (very numerous). Among the young-wood specimens (fig. 12) the species means average even higher, as in *C. stricta* (280), *C. equisetifolia* (129), and *C. cunninghamiana* (377).

Vessel distribution is universally solitary (figs. 1, 2, 4, 12). In general, *Casuarina* is diffuse-porous, but in nearly all species which show indefinite growth rings there is a slight tendency toward a manifestation of ring-porosity (figs. 1, 10, 26). Most specimens of *C. stricta* and *C. glauca* exhibit this tendency, but some may be strictly diffuse-porous; *C. deplancheana*, *C. nodiflora*, and *C. sumatrana* (fig. 2) are typically diffuse-porous more often than not.

In transverse sections of the old wood (far from the pith) the vessels or pores are typically circular to oval in outline (figs. 4, 11, 13) in the majority of the species, but in *C. inophloia*, *C. muelleriana*, *C. collina*, and *C. lepidophloia*

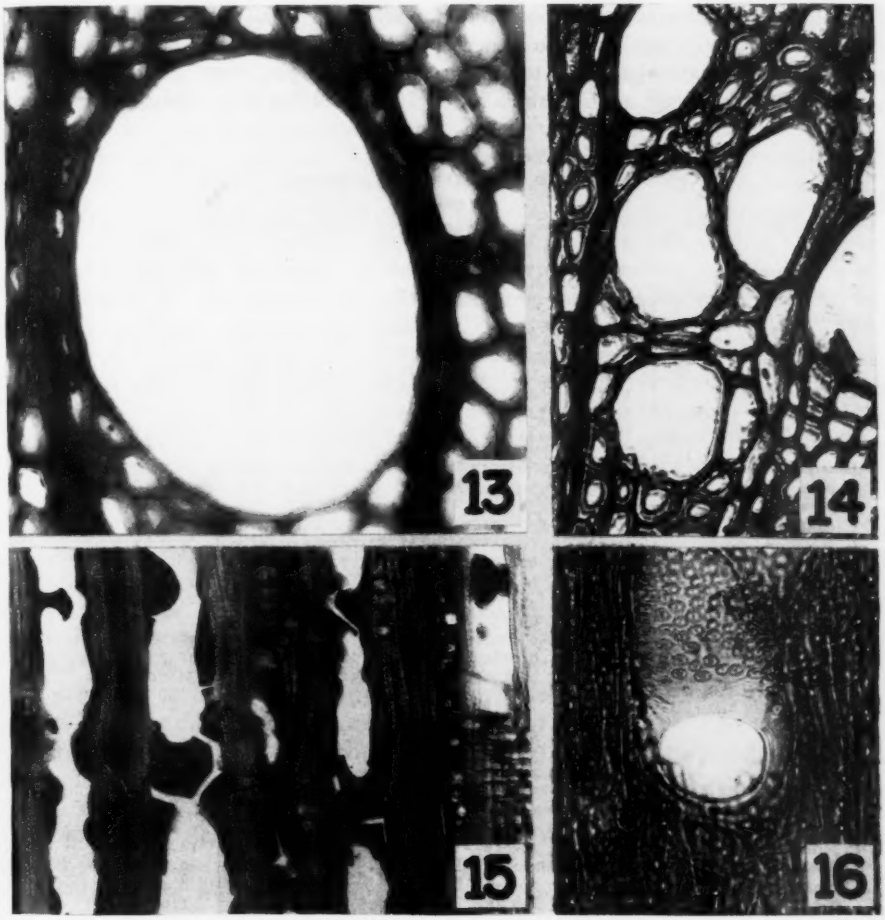
TABLE 1

NUMBER OF VESSELS PER SQUARE MILLIMETER IN OLD WOOD

Species	Range	Most frequent range		Means		Number of specimens
Leiopitys:						
C. stricta	10– 69	27– 54	N–VN*	41	VN	7
C. glauca	15–115	25– 30	N–VN	44	VN	8
C. junghuhniana	14– 31	18– 25	MN– N	22	N	1
C. equisetifolia	4– 23	5– 15	MF–MN	12	MN	11
C. suberosa	14–173	20–100	N– VN	75	VN	13
C. cunninghamiana	15–104	26– 66	N– VN	36	N	11
C. distyla	38– 89	45– 80	VN	65	VN	2
C. torulosa	9– 48	19– 34	MN– N	19	MN	11
C. decaisneana	6– 21	10– 18	MN	11	MN	1
C. cambagei	37– 79	41– 64	VN	58	VN	3
C. dialsiana	37– 90	52– 71	VN	63	VN	1
C. fraseriana	6– 36	15– 30	MN– N	17	MN	6
C. lepidophloia	25– 35	28– 32	N	29	N	1
C. collina	30– 52	34– 45	N–VN	40	N	2
C. montana	8– 25	10– 23	MN– N	17	MN	2
C. deplancheana	5– 10	8– 9	MF	8	MF	1
C. poissoniana	5– 10	7– 9	MF	8	MF	1
C. sumatrana	4– 18	6– 12	MF	8	MF	3
C. nodiflora	4– 17	7– 14	MF–MN	8	MF	3
Trachypitys:						
C. nana	33– 99	52– 77	VN	67	VN	1
C. humilis	19– 84	36– 67	N– VN	60	VN	1
C. inophloia	32–263	36–189	N– VN	82	VN	3
C. luehmanni	23– 54	30– 42	N– VN	33	N	3
Acanthopitys:						
C. bicuspidata	242–369	284–348	VN	304	VN	1
C. acutivalvis	129–369	221–295	VN	262	VN	1
C. baxteriana	82–306	158–232	VN	180	VN	1
C. muelleriana	179–432	221–369	VN	299	VN	1
		Average of means 66.7 Number of specimens 100				

* MF = moderately few; MN = moderately numerous; N = numerous; VN = very numerous.

they tend to be somewhat angular. The smaller vessels of young wood (close to the pith) are typically angular (fig. 14). The vessel elements have "tails" of various sizes (fig. 18), but these are usually visible only on macerated specimens. The vessel walls are unevenly thickened and vary among the species from 1 to 10 μ in thickness, and most frequently from 2.0 to 5.5 μ. Using 6 μ arbitrarily to separate thin from thick walls, twenty species have mostly thin vessel walls (figs. 4, 13), while seven species have mostly thick walls. *C. collina* and *C. inophloia* have a higher percentage of vessels with thick walls than have other



FIGS. 13–16.—Fig. 13, *C. cunninghamiana:* transverse section to show circular to oval, thin-walled (about 2.5 μ thick) vessel of old wood, and thick-walled tracheids and fiber-tracheids. ×500. Fig. 14. *C. montana:* transverse section to show angular outline of vessels in young wood. Notice narrow metatracheal parenchyma and gelatinous fiber-tracheids. ×415. Fig. 15, *C. stricta:* radial section showing gum deposits in vessels, and metatracheal parenchyma. ×100. Fig. 16, *C. muelleriana:* radial section illustrating simple perforation plate in end wall of vessel element. ×415.

species. The walls of vessels in young wood vary from less than 1 to 6 μ and are usually less than 4 μ.

The cross-sectional diameter of vessels in old wood ranges from extremely small (fig. 10), as in *C. muelleriana* (range 11–54 μ), to moderately large (fig. 2), as in *C. sumatrana* (range 15–205 μ). The range of species means in old wood (table 2) demonstrates a variation from very small (5 spp.) (figs. 4, 11) to moderately small (19 spp.) (figs. 4, 11) to medium-sized (3 spp.) (fig. 2). *C. corniculata* and *C. paludosa* are excluded from this statement as the samples of these are of young wood. The average of means for the genus is 71.0 ± 0.4 μ (standard deviation 35.7 ± 0.3) (moderately small). A transverse section of *C. glauca* (fig. 11) presents an average picture of the genus with respect to vessel size (most frequent range 45–90 μ, mean for the species 63 μ, moderately small). Vessel diameters in young wood (fig. 12) are consistently smaller, ranging from extremely small (figs. 12, 28), as in *C. equisetifolia* (range

TABLE 2

VESSEL DIAMETERS IN OLD WOOD
(In microns)

Species	Range	Most frequent range	Means	Standard deviation of mean	No. of specimens
Leiopitys:					
C. stricta	18–127	40– 80 VS–MS*	56±0.7 MS	20.3±0.6	7
C. glauca	12–140	45– 90 VS–MS	63±0.9 MS	24.7±0.6	8
C. junghuhniana	21–131	84–115 MS– M	92±2.7 MS	26.5±1.9	1
C. equisetifolia	18–168	43–145 VS– M	87±0.9 MS	31.4±0.7	11
C. suberosa	12–199	31–146 VS– M	66±0.8 MS	26.7±0.6	12
C. cunninghamiana	15–168	35–115 VS– M	75±0.9 MS	30.6±0.7	10
C. distyla	18– 99	46– 68 VS– M	55±1.4 MS	19.4±0.9	2
C. torulosa	15–168	60–157 MS– M	94±1.1 MS	32.2±0.8	9
C. decaisneana	26–153	70–109 MS– M	82±3.4 MS	34.4±2.4	1
C. cambagei	18– 96	37– 70 VS–MS	51±0.8 MS	14.4±0.6	3
C. dialsiana	15– 92	57– 74 MS	58±1.8 MS	17.6±1.2	1
C. fraseriana	23–184	80–148 MS– M	95±1.4 MS	35.3±1.0	6
C. lepidophloia	26–116	60– 89 MS	74±1.9 MS	18.8±1.3	1
C. collina	15–128	66– 95 MS	69±1.7 MS	23.3±1.2	2
C. montana	17–118	43– 84 VS–MS	64±1.5 MS	21.6±1.1	2
C. deplancheana	43–155	96–135 MS– M	109±2.5 M	25.0±1.8	1
C. poissoniana	20–137	63–107 MS– M	80±2.8 MS	28.3±2.0	1
C. sumatrana	15–205	94–184 MS– M	162±2.4 M	40.7±1.7	3
C. nodiflora	37–199	99–160 MS– M	122±2.0 M	34.9±1.4	3
Trachypitys:					
C. nana	15–107	38– 61 VS–MS	52±1.7 MS	17.2±1.2	1
C. humilis	23– 84	38– 54 VS–MS	46±1.8 VS	17.7±1.3	1
C. inophloia	20–107	31– 76 VS–MS	53±1.1 MS	19.7±0.8	3
C. luehmanni	20–130	50– 92 MS	73±2.7 MS	26.7±1.9	1
Acanthopitys:					
C. bicuspidata	14– 89	29– 52 VS–MS	45±1.5 VS	14.8±1.1	1
C. acutivalvis	17– 57	20– 47 ES– VS	34±1.0 VS	9.7±0.7	1
C. baxteriana	17– 61	24– 40 ES– VS	33±0.8 VS	8.4±0.6	1
C. muelleriana	11– 54	23– 46 ES– VS	32±1.0 VS	1.0±0.7	1

Average of means for the genus: 71.1±0.4 MS (standard deviation 35.7±0.3)
Total number of specimens: 94
Total number of measurements: 9,375

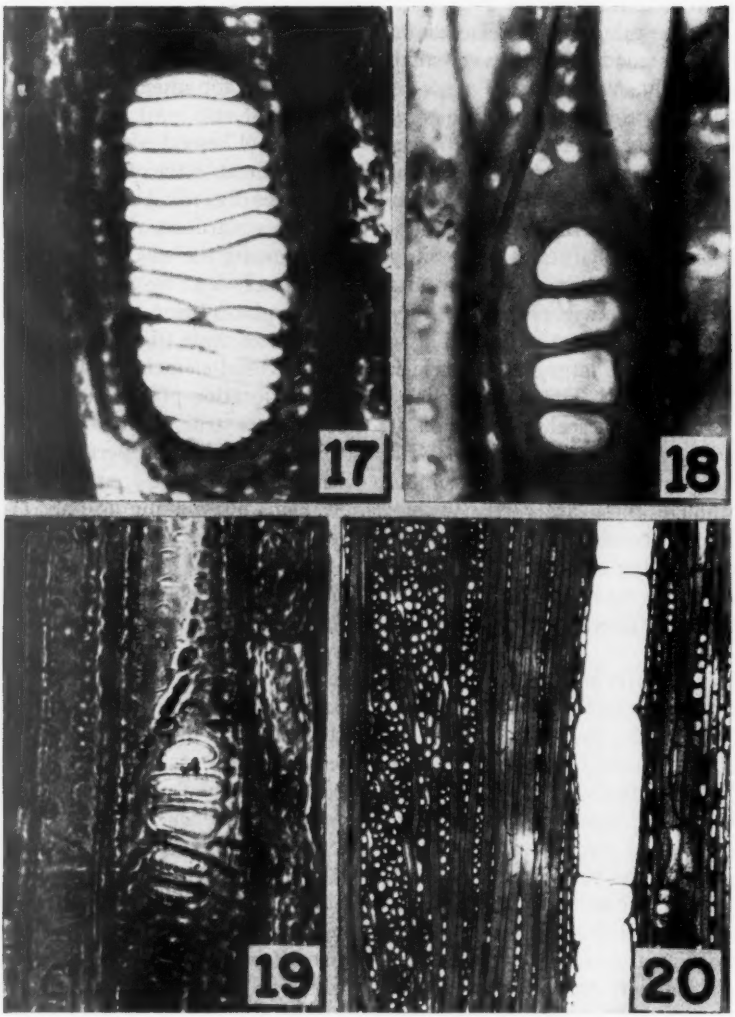
* ES = extremely small; VS = very small; MS = moderately small; M = medium-sized.

8–99 μ), to medium-sized, as in *C. nodiflora* (range 31–148 μ). Most vessel diameters of young wood fall in the very small and moderately small classes.

Tyloses are abundant but are very difficult to study, as are all the parenchymatous tissues, owing to the abundance of dark-colored "gum" deposits which obscure their structure even in unstained sections. Tyloses appear fairly early and may often be seen in the young wood of a young twig. They are very variable in size, often only 2–3 μ in diameter, forming large masses which block the vessels. They are usually spherical to subspherical, thin-walled at first, living, and often associated with "gum" deposits. The cells have simple pits, although these are rarely visible. Later, during the conversion of sapwood to heartwood, the cells become lignified and probably eventually lose their protoplasm. No truly sclerotic tyloses were observed.

"Gum" deposits are frequent; in fact, their appearance in the vessels seems to be the first indication, even before the formation of tyloses, of the conversion of sapwood to heartwood. The "gum" is apparently exuded from wood parenchyma into the vessels, forming semispherical globules along the vessel walls (fig. 15). These globules eventually coalesce to form a layer covering the wall. "Gum" plugs blocking the vessels are formed in a similar manner, especially where the vascular rays come in contact with the vessels (fig. 15). Frequently, in heartwood, many of the fibrous elements are filled with "gum." The term "gum" is used as defined by the Committee on Nomenclature of the International Association of Wood Anatomists (**37**). It is doubtful, however, whether the term has any chemical significance. Tannin is present in the deposits as evidenced by chemical tests with iron salts, but resins are absent as shown by a negative aqueous cupric acetate reaction (**78**). No adequate tests for mucilages or gums are known (**78**).

In the old wood of eight species the vessel elements have only simple perforation plates (fig. 16). Although the proportion of scalariform perforation plates varies among the specimens, ten species are characterized by 1–40% of the perforation plates being scalariform and the remainder simple (fig. 17). Usually, the smaller vessel elements bear the scalariform perforation plates, although this is not always true. Three species have scalariform and simple perforation plates, with the former type appearing in all the specimens but making up only 1–5% of the total number. The vessel elements of *C. stricta* and *C. distyla* are typified by simple perforation plates, but occasional specimens of old wood may show scalariform perforation plates up to 15%. On the contrary, *C. equisetifolia*, *C. fraseriana*, *C. torulosa*, and *C. sumatrana*, characterized by 1–30% of the vessel elements bearing scalariform perforation plates, occasionally show wood with only simple perforation plates. In the nineteen species (excluding *C. paludosa*, of which only a young specimen was available) with scalariform perforation plates, the number of bars ranges from two to thirty (few to many). Two species have mostly the few to intermediate number of bars, fourteen have mostly the intermediate number (fig. 17), and three mostly the intermediate to many. *C. glauca* often shows scalariform plates in which several of the bars are vestigial. Of the nineteen species (excluding *C. paludosa*) with scalariform plates, fourteen have bars with openings classified as mostly narrow and five with openings mostly narrow to wide. This classification



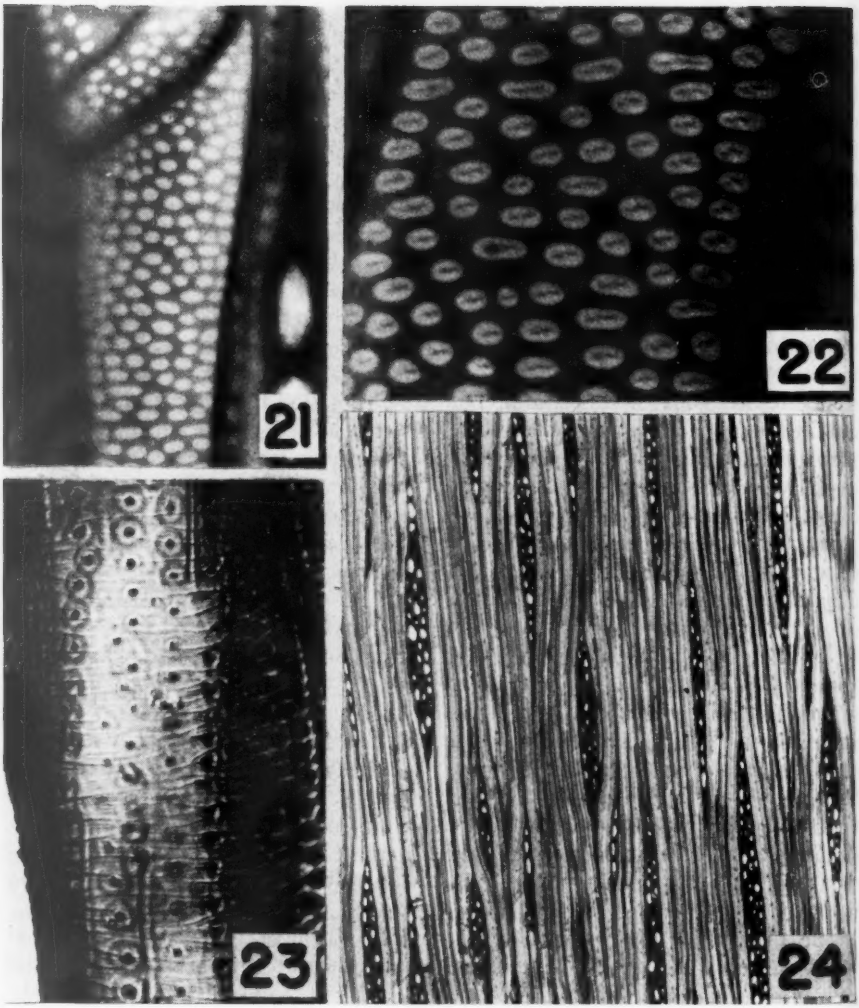
FIGS. 17–20.—Fig. 17, *C. stricta:* radial section illustrating scalariform perforation plate with intermediate number of bars. ×850. Fig. 18, *C. stricta:* radial section from young wood to show small, scalariform perforation plate with few bars. Notice "tail" of vessel element. ×1000. Fig. 19, *C. muelleriana:* radial section from wood near pith illustrating small, oval, scalariform perforation plate with few bars and circular bordered pit-pairs in walls of tracheary elements. ×800. Fig. 20, *C. sumatrana:* tangential section demonstrating tall rays, transverse and oblique end walls on vessel elements, tyloses, small aggregate ray, and thick-walled fiber-tracheids. ×75.

refers to the most frequent range of opening widths; most of the species classified as narrow, however, may have occasional bars with openings as wide as 10–12 μ (13.5 μ in *C. sumatrana*). In all the species with scalariform and simple perforation plates, the scalariform plates have bars with complete borders. The young wood of sixteen species, including *C. corniculata* and *C. paludosa*, has been studied. The vessel elements in twelve of the species have scalariform and simple perforation plates, the former type often accounting for 5% to as high as 70% of the plates. Three species have 1–20% of the perforation plates scalariform, but certain specimens may show exclusively simple perforation plates. *C. corniculata* has only simple perforation plates. It is noteworthy that scalariform perforation plates are present in the young wood of *C. nana*, *C. humilis*, *C. inophloia*, and *C. muelleriana*, but entirely absent in the old wood of the same species. There is little difference to be noted in the scalariform perforation plates of young wood as to number of bars or width of perforations. Similar counts have been recorded for both old and young specimens; but, in general, the plates in the young wood fall in the few to intermediate class for number of bars and in the narrow class for width of perforation (figs. 18, 19).

The angle which the end walls of the vessel elements make with the lateral walls in old wood varies from 20° to 90°, with 40°–80° as the predominating range (figs. 7, 20, 21). Nevertheless, in twenty-six species there are some vessel elements with the end walls at 90° (fig. 20). In *C. stricta*, the end walls vary from 25° to 85° but are never completely transverse. In the young wood of ten species the end walls on the vessel elements range from 15° to 85°, mostly 25°–70°. In six species the young wood may have a few vessel elements with end walls at 90°.

In the old wood the intervascular pitting is largely alternate. In one species, *C. nodiflora*, the intervascular pitting on the walls of perhaps two-thirds of the vessel elements is alternate, but many of the smaller vessel elements show definite transitional and opposite pitting. In the other species the intervascular pitting is overwhelmingly alternate (figs. 22, 23); but opposite pitting occurs, however, occasionally in the walls of vessels in nearly all the species—more commonly in *C. dialsiana*, *C. fraseriana*, *C. deplancheana*, *C. stricta*, and *C. suberosa* (fig. 21). The intervascular pit-pairs are abundant and crowded, except in *C. nodiflora*, where they are often sparse. The size of the pit-pairs varies from 2.0 to 16.5 μ but very rarely reaches 6.5 μ in diameter. The most frequent range is from 2.5 to 6.0 μ (minute to small). Three species have pit-pairs up to 10.0 μ in diameter; and in *C. nodiflora* the elongate, transitional pit-pairs may reach 16.5 μ (very large). The shape of the intervascular pit-pairs is correlated with their size. They are mostly circular, to oval, but occasionally elongate. Of the sixteen species with young wood examined, eight species have alternate intervascular pitting, five have mostly alternate but some opposite, and three (*C. suberosa*, *C. nodiflora*, and *C. paludosa*) have abundant transitional pitting near the pith, with a proportional increase of opposite and alternate in slightly later wood. The shape and size varies much as in the old wood.

The pitting between vessels and vascular rays in old wood is mostly alternate but with some transitional to opposite in two species, mostly alternate with some opposite in five species, and wholly alternate in twenty-one species. The size of the pit-pairs varies from 0.7 (fine) to



FIGS. 21–24.—Fig. 21, *C. suberosa:* tangential section demonstrating opposite to alternate intervascular pitting, and oblique end walls on vessel elements. Notice that pit-pairs on vasicentric tracheids are equal to or larger than corresponding pit-pairs on vessel side-wall. ×500. Fig. 22, *C. suberosa:* tangential section to show alternate intervascular pitting. ×1000. Fig. 23, *C. suberosa:* radial section to demonstrate spiral thickenings in vessel element and alternate intervascular pitting. ×800. Fig. 24, *C. equisetifolia:* tangential section demonstrating old wood without aggregate rays, heterogeneous IIB rays with tendency for elimination of uniseriate rays, and thick-walled fiber-tracheids. ×100.

10 μ (medium), being mostly 2.0–4.0 μ (fine). The pit-pairs are half-bordered, circular to oval, occasionally elongate, and abundant. In the young wood of sixteen species the ray-vessel pitting is mostly alternate, with some transitional to opposite in four species, mostly alternate with some opposite in four species, and alternate in eight species. Other characteristics of the pit-pairs are similar to those found in the old wood.

Measurements of the length of vessel elements were made from macerated material of five specimens of *C. suberosa* and from one specimen of each of twenty-five other species. Such measurements were not made for *C. poissoniana*, *C. corniculata*, and *C. paludosa*. The most frequent range of vessel-element lengths in microns for the five specimens of *C. suberosa* are: 247–416, 381–526, 450–599, 456–611, and 492–738. The means for one hundred measurements in each of the same five specimens are: 339, 455, 533, 520, and 571 μ. From this survey, it is obvious that the variation in vessel-element length in this species is even greater than the variation in fiber lengths. Little value at the species level, therefore, can be placed on the measurements of a hundred vessel elements from only one specimen of each of the other twenty-five species. Considering the genus as a whole, therefore (thirty specimens, three thousand measurements), the range of vessel-element lengths is from 190 (very short) to 1275 μ (very long). The most frequent range of lengths is from 276 (moderately short) to 591 μ (medium-sized), and the mean for the genus is 439 ± 3.3 μ (standard deviation 182.2 ± 2.4) (medium-sized).

Spiral thickenings are characteristically present in all or some of the vessels of old wood in thirteen species (fig. 23), are characteristically absent but may appear sporadically in occasional specimens in five species, and are characteristically absent in nine species. In nine of the thirteen species characterized by the nearly constant presence of spiral thickenings in the vessels, the thickenings also appear in the fibrous elements (table 3). In all species in which spiral thickenings on vessel walls are sporadic in appearance or absent, they are absent from the fibrous elements. There is no constant correlation between the presence of spiral thickenings in the vessels and the presence of simple perforation plates, although the two structures are associated in eight of the thirteen species. There is no such correlation between the two in the young wood. It may be noticed, however, that, as in old wood, there is a similar correlation for the presence of spiral thickenings in both types of elements. Six of the sixteen young specimens show a nearly constant presence of spiral thickenings in the vessels, six demonstrate a sporadic appearance, and four have no spiral thickenings.

VASCULAR RAYS.—The vascular rays (fig. 10) are abundant in old wood, ranging from five to eighteen rays per millimeter, counted tangentially in tangential sections. The most frequent range is eight to twelve. The number in young wood tends to be higher, between nine and fourteen. The rays are heterogeneous IIB throughout the genus (figs. 3, 5, 24). In certain species there is a trend toward the elimination of one type of ray. Aggregate rays are absent from, and the smaller multiseriates predominate in, the old wood of *C. equisetifolia* (fig. 24) and *C. cambagei*. This partial elimination of the uniseriate rays is associated with a trend toward the homogeneous type I condition. By the elimination of most of the multiseriate rays, there is a tendency toward the heterogeneous type III rays in

the old wood of *C. inophloia*, *C. sumatrana*, and occasionally in *C. torulosa*; aggregate rays are still present, but, of the smaller rays, the uniseriates predominate (fig. 25). For reasons to be explained later, the aggregate ray is not included in the consideration of the width and height of the rays. In the old wood of twenty species, the rays in tangential aspect may be from one to ten cells wide. In seven species the rays may be from one to four cells wide. Regardless of the maximum widths, however, the most frequent width is from one to two in fifteen species, from one to three in eight species, from one to four in one species, and from two to three in one species. In the young wood the rays approximate the width of the rays in the old wood of the same species, although in four species they tend to be wider and in two species more narrow. In the old wood of five species the uniseriate rays vary from one to fifteen cells in height (most frequent range five to twelve); in thirteen species, from one to twenty-five (most frequent range eight

TABLE 3

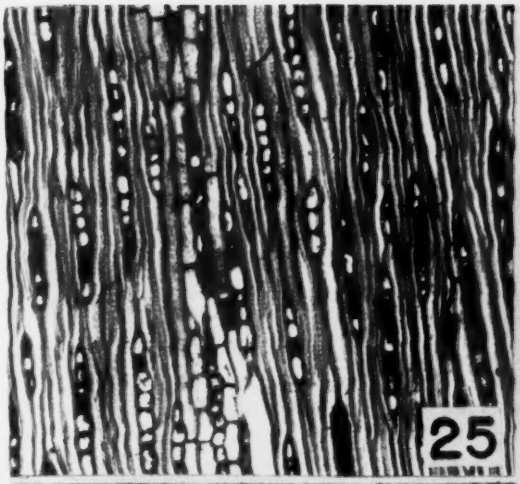
ASSOCIATION OF SPIRAL THICKENINGS WITH PERFORATION PLATE TYPE

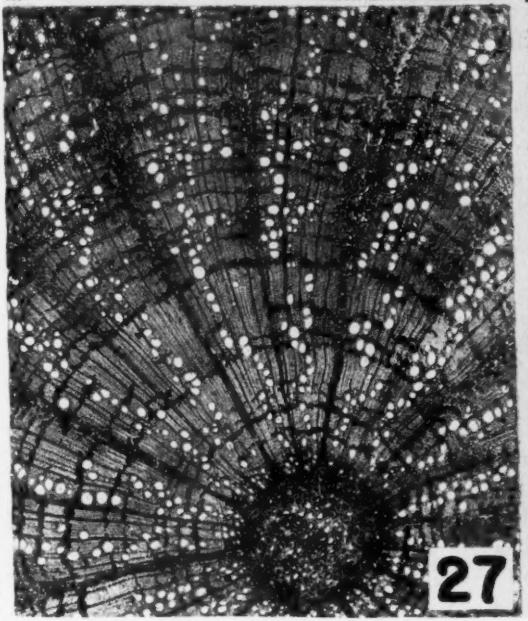
Sections and species	Spiral thickenings on vessel elements	Spiral thickenings on fibrous elements	Scalariform and simple perforation plates	Simple perforation plates only	Spiral thickenings on vessel elements	Spiral thickenings on fibrous elements	Scalariform and simple perforation plates	Simple perforation plates only
Leiopitys:								
C. stricta	s*	−†	+‡		s	s	+	
C. glauca	s	−	+		−	−	+	
C. junghuhniana	−	−	+					
C. equisetifolia	s	−	+		−	−	+	
C. suberosa	+	+	+		s	+	+	
C. cunninghamiana	s	−	+		s	−	+	
C. distyla	−	−	+		s	−	+	
C. torulosa	+	s	+		s	−	+	
C. decaisneana	−	−	+					
C. cambagei	+	+	+					
C. dialsiana	−	−	+					
C. fraseriana	−	−	+		−	−	+	
C. lepidophloia	+	−	+					
C. collina	−	−	+					
C. montana	−	−	+		−	−	+	
C. deplancheana	−	−	+					
C. poissoniana	−	−	+					
C. sumatrana	s	−	+					
C. nodiflora	+	−	+		s	−	+	
Trachypitys:								
C. nana	+	+		+	+	+	+	
C. humilis	+	+		+	+	+	+	
C. inophloia	+	+		+	+	+	+	
C. luehmanni	+	s		+				
Acanthopitys:								
C. bicuspidata	+	+		+				
C. acutivalvis	+	+		+				
C. baxteriana	+	+		+				
C. muelleriana	+	+		+	+	+	+	

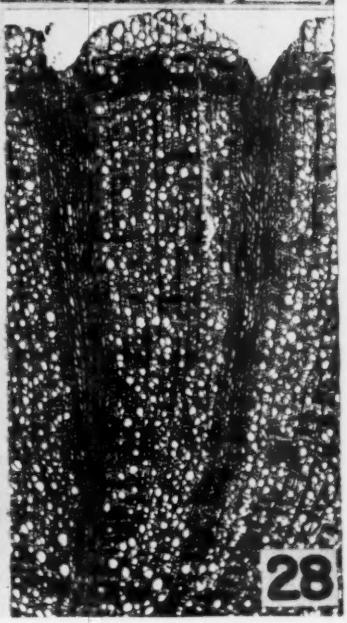
* s = Spiral thickenings generally absent but may appear sporadically in certain specimens.

† − = Spiral thickenings characteristically absent.

‡ + = Spiral thickenings characteristically present on all or part of the elements.



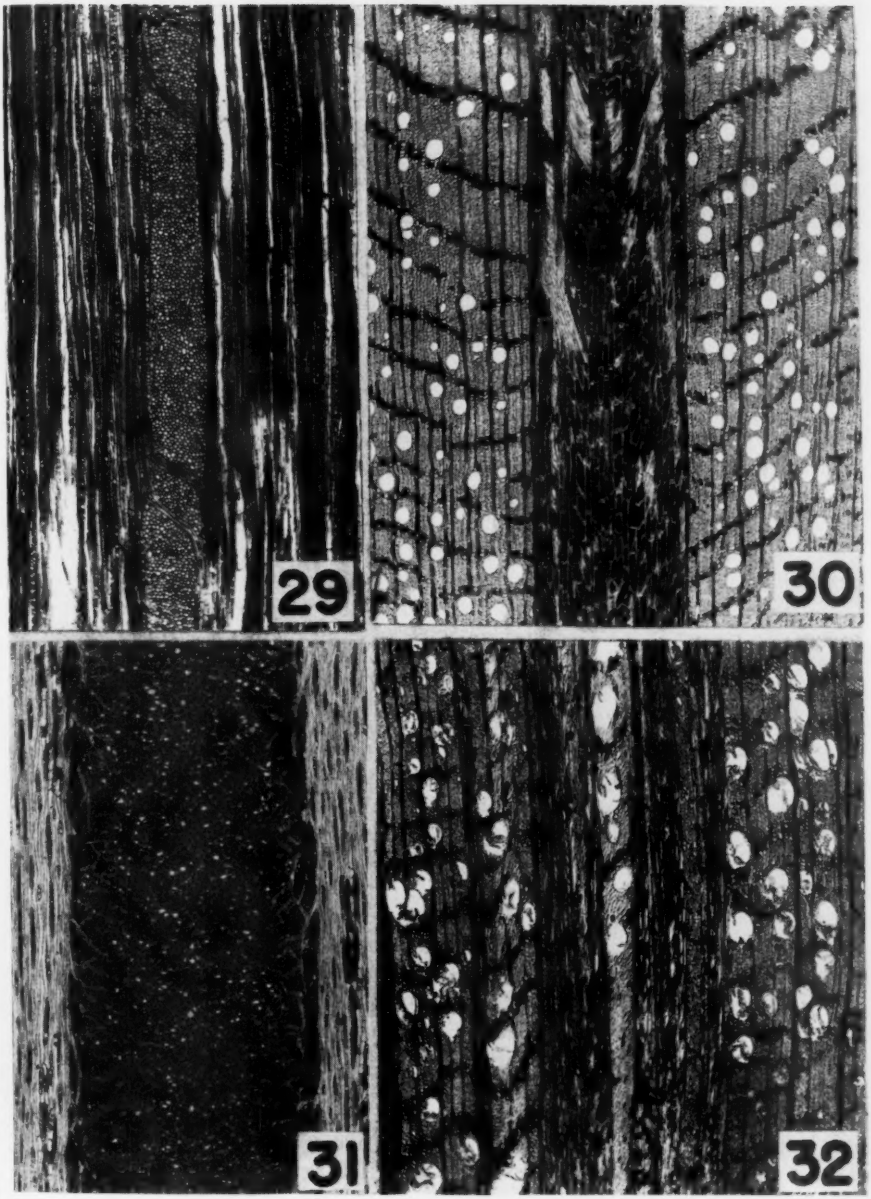




FIGS. 25–28.—Fig. 25, *C. inophloia:* tangential section demonstrating wood with tendency for loss of ordinary multiseriate rays. Notice wood parenchyma in tangential view. ×100. Fig. 26, *C. stricta:* transverse section to show origin of aggregate rays by widening of uniseriates and multiseriates and aggregation of both types. Notice diffuse and narrow and coarse metatracheal parenchyma; indefinite growth rings; and tendency to ring-porosity. ×37. Fig. 27, *C. stricta:* transverse section to demonstrate widening of uniseriate and multiseriate rays and aggregation of these to form aggregate rays. Notice also parenchyma which is narrow to coarse in transverse section, and indefinite growth rings. ×37. Fig. 28, *C. cunninghamiana:* transverse section demonstrating small, nearly compound or compound rays in young stem; very numerous, extremely small to moderately small vessels; and retardation of growth in region of aggregate rays. ×37.

to fourteen); in seven species, from one to thirty-five (most frequent range eight to fifteen); and in one species, from one to fifty (most frequent range nine to twenty) (fig. 20). In young wood the ranges tend to be higher; species with uniseriate rays one to twenty and one to thirty cells high are common. The most frequent range is from eight to fifteen. The multiseriate rays in old wood are commonly higher than the uniseriates. In one species the multiseriate rays vary from four to fifteen (most frequent range seven to twelve), in one species from four to twenty-five (most frequent range six to twelve), in sixteen species from four to fifty (most frequent range ten to twenty-two), and in nine species from four to one hundred cells high (most frequent range twelve to twenty-five). The multiseriate rays in young wood tend to be higher. The most frequent range is from ten to thirty cells high; but rays often achieve heights of thirty-five, sixty, ninety, and, in *C. nodiflora*, one hundred and twenty-five cells.

In this study the aggregate ray (figs. 26, 27, 32–35) is considered a structure separate and fundamentally different from the more ordinary uniseriate and multiseriate rays, in opposition to BARGHOORN (16). The aggregate ray in *Casuarina* is clearly formed, ontogenetically, in most cases by an aggregation of both nondiverging uniseriate and multiseriate rays. Uniseriate rays may be included directly in the formation, or they may first widen into multiseriates (fig. 26, 27). This formation of the aggregate ray may be seen repeatedly in the young stems of *Casuarina* (figs. 12, 27). This sequence of development suggests that the aggregate ray in *Casuarina* was not derived phylogenetically from a large multiseriate ray which became dissected (12, 16) but evolved through the aggregation of smaller rays (4, 74). Instances may be cited, however, in the young wood of *C. equisetifolia*, in which wide multiseriate rays and small compound rays arise directly by the widening of a uniseriate ray or arise in the first increment of the secondary xylem. *C. equisetifolia*, however, very rarely possesses aggregate rays in the old wood (figs. 4, 24) and is characterized by early dissection of the small aggregate and compound rays to produce the "diffuse" ray condition. Typical aggregation is observable in the other fifteen species with young wood. The young wood of *C. cunninghamiana* occasionally presents conditions similar to the compound-ray formation of *C. equisetifolia*. The aggregate rays in young twig wood are often narrow and relatively long in a vertical direction and evidently develop in relation to the leaf traces. The latter may often be seen in tangential sections surrounded by the aggregate rays. As noted by BAILEY (4), in a few species "nearly compound" to truly compound rays are developed from aggregate rays (figs. 28, 29) but are replaced by aggregate rays or by "diffuse" rays in older wood farther from the pith. The large rays in the old wood of *C. inophloia* (figs. 30, 31), *C. fraseriana*, and *C. suberosa*, however, may be classed as "nearly compound" as the rays are often composed almost entirely of parenchyma. Once formed in the young wood near the pith, the aggregate ray may increase in width by a gradual widening and by the inclusion of additional multiseriate rays. In the opinion of this author, the smaller rays which compose the larger aggregate completely lose their identity and become interwoven with each other into a massive parenchymatous ray in which the radial rows of fibrous elements originally separating the individual rays in younger wood wind and twist in various

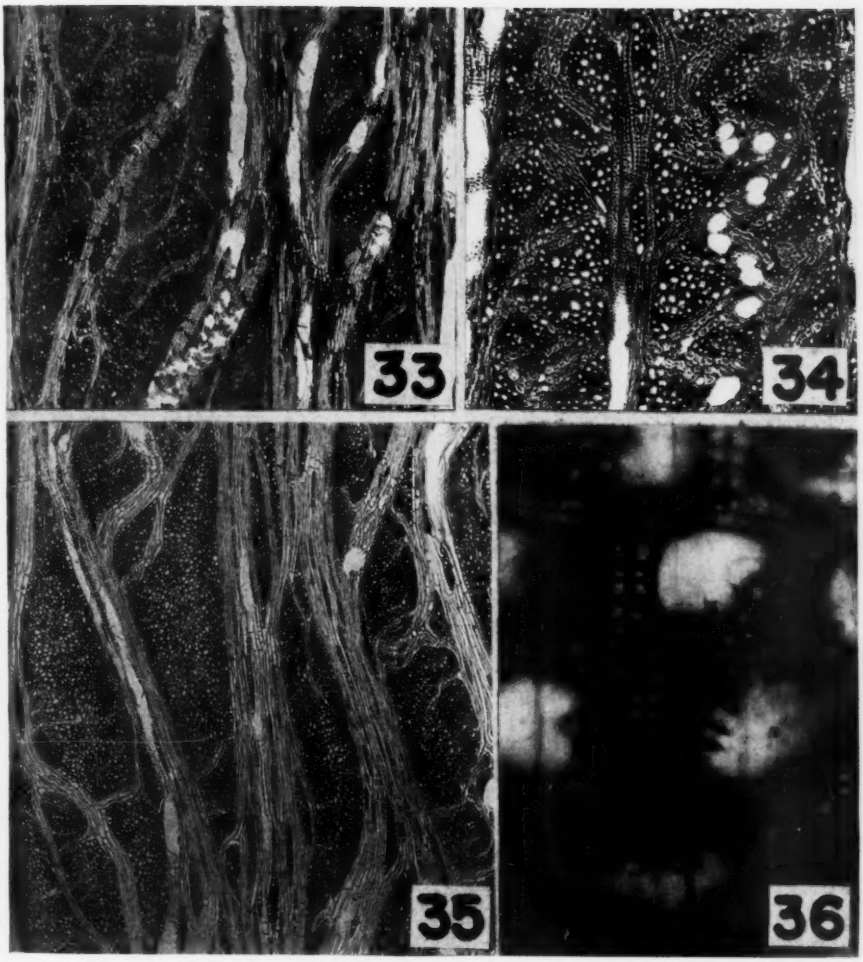


FIGS. 29–32.—Fig. 29, *C. cunninghamiana:* tangential section demonstrating small compound or nearly compound rays in young stem. ×37. Fig. 30, *C. inophloia:* transverse section showing large aggregate ray to nearly compound ray. ×37. Fig. 31, *C. inophloia:* tangential section showing large aggregate to nearly compound ray. ×37. Fig. 32, *C. glauca:* transverse section to show aggregate rays in process of disintegration and numerous to very numerous vessels. ×37.

directions (figs. 34, 35), even in the horizontal plane (fig. 34). A tangential view of a large aggregate ray gives a better conception of its structure as a large parenchymatous ray in which the fibrous elements, "captured" in the ontogeny of the ray, are turned aside and are redirected variously. It is the presence of the fibrous elements which designate it as an aggregate ray, however, and as a structure different from the smaller multiseriates and derived ontogenetically, and probably phylogenetically, by a process of aggregation of smaller rays, and not from a multiseriate ray.

Twenty-three species have aggregate rays in the old wood. The variation in size is very great (figs. 20, 30–35), and no general size limits can be stated. The variation in width in old wood is from 0.06 to 4.0 mm. and in length from 0.1 to 50 mm. or more. This latter measurement is conservative, for in *C. fraseriana*, *C. inophloia*, *C. luehmanni*, and *C. sumatrana* the aggregate rays were longer than the available specimens of wood; and, therefore, the recorded measurement does not give a true conception of the great length of some of the aggregate rays in these species. It is difficult to give a most frequent range, but in woods with average aggregate ray development the range is from 0.1 to 0.5 mm. in width and from 5.0 to 15 mm. in length. The old wood often demonstrates rays still in the process of development, but nearly all species with aggregate rays in the old wood show the rays in the process of dissection (figs. 32, 33). This process is evident, especially in transverse section, where it may be observed that the large rays are split up into smaller aggregates and multiseriates by a divergence associated with an increase in the amount of fibrous elements. The dissection of the aggregate ray may be imaginatively envisioned as occurring by a splitting of the ray in ontogeny by the intervention of fibrous elements arising possibly as described by BARGHOORN (**15**): by the loss of ray initials and by the transformation of ray initials to fusiform initials in the cambium.

It is the opinion of the author, then, that the aggregate ray in *Casuarina* is a specialized structure and arises ontogenetically in most species, and probably has arisen phylogenetically, by an aggregation of smaller rays. This is in disagreement with the idea that an aggregate ray is a stage in the disintegration of a wide multiseriate ray (**12, 16**); but it is in agreement with the earlier conception of the aggregate ray in *Casuarina* (**4, 74**). It is obvious, however, that the aggregate ray is not a permanent structure but is dissected eventually in a number of species as later wood is formed (figs. 32, 33). The dissection occurs at different growth stages among the species. Four species (*C. decaisneana*, *C. baxteriana*, *C. cambagei*, and usually *C. equisetifolia*) do not have aggregate rays in the old wood (figs. 4, 24). Aggregate rays, usually found in the old wood of *C. glauca* and *C. junghuhniana*, may become dissected so that an occasional specimen shows only smaller rays, as in the four species mentioned above. The wood of *C. acutivalvis* shows very small aggregate rays less than a millimeter long and in the last stages of dissection. The rays in this species have nearly attained the "diffuse" condition. Young-wood specimens of *C. equisetifolia*, however, possess small aggregate and small compound (or wide multiseriate) rays which are quickly dissected, giving rise to wood with "diffuse" multiseriate and uniseriate rays. Young specimens of the other four species were not available. The "diffuse" ray condition of these species does support



FIGS. 33–36.—Fig. 33, *C. glauca:* tangential section showing aggregate rays in process of disintegration. Notice broad strands of fibrous and vessel elements. ×37. Fig. 34, *C. collina:* tangential section to show "collina type" of aggregate ray in which fibrous elements are oriented vertically and horizontally (directed radially and tangentially). Fibrous elements form lattice-like structure. Notice crystals in some of ray cells. ×85. Fig. 35, *C. cunninghamiana:* tangential section illustrating large aggregate rays in process of disintegration. This is an example of the "cunninghamiana type" of aggregate ray in which vertical and nearly vertical fibers form an anastomosing framework. ×37. Fig. 36, *C. nodiflora:* radial section to illustrate greater lignification of ray cells found in certain species. ×500.

the thesis, however, that smaller multiseriate rays may arise in *Casuarina* from aggregate rays by the dissection of the latter (**12, 16, 128**).

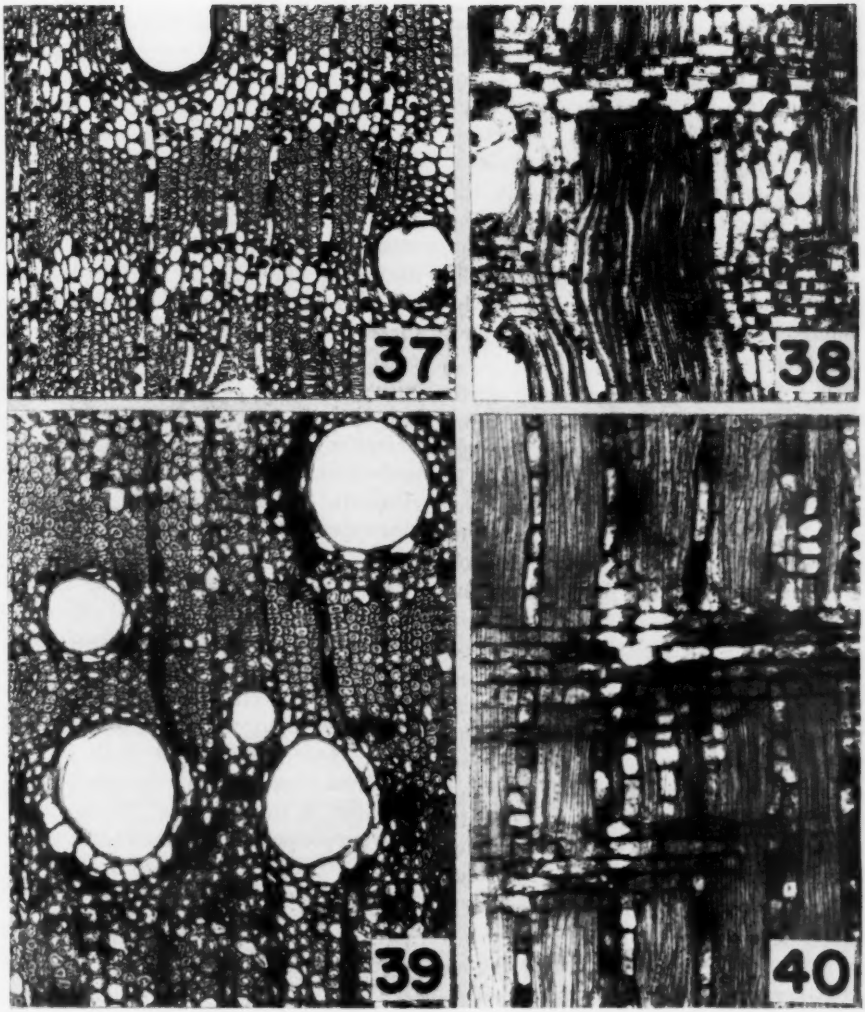
It is interesting to note the depression in the outline of the surface of the wood owing to the retardation of growth in the region of the aggregate rays (fig. 28) similar to that described for *Quercus* by BAILEY (**5**).

The simple pit-pairs between ray cells and xylem parenchyma cells in both young and old wood vary from less than 1 μ to 4 μ in diameter. The most frequent size is from 1.5 to 3.5 μ. The ray-parenchyma pit-pairs are many and are very frequently clustered in groups of three to five. The ray cells become lignified very early in young wood and are prominently lignified in old wood (fig. 36). Crystals of the solitary, rhombohedral type, probably calcium oxalate, are very common in the rays of all types (fig. 34) and often appear even in the young wood. Thick walls are often deposited in the ray cells containing the crystals, and these may be considered as sclerotic ray cells. More typical sclerotic ray cells with ramiform pits but without crystals were found in the old wood of eighteen species. They occur more commonly in the aggregate rays. The deposition of crystals in the rays and wood parenchyma cells and the development of sclerotic ray cells are prominent features in the conversion of sapwood to heartwood.

WOOD PARENCHYMA.—In all species wood parenchyma is abundant. Apotracheal parenchyma of the diffuse and metatracheal types is predominant in both young and old wood. There is a strong tendency for the elimination of the diffuse type. No species has only diffuse parenchyma, but all have metatracheal (figs. 4, 5, 11, 15, 25). Diffuse parenchyma strands are never abundant but may be classed as few or rare. In old wood, six species have diffuse with narrow metatracheal parenchyma (fig. 2). Twelve species have diffuse with narrow and coarse, mostly narrow, metatracheal parenchyma (figs. 4, 5, 10, 11); five species have diffuse with narrow and coarse, mostly coarse, metatracheal parenchyma (figs. 26, 27); one species has no diffuse but narrow metatracheal parenchyma only; two species have no diffuse but narrow to coarse, mostly narrow, metatracheal parenchyma; and one species has no diffuse but narrow to coarse, mostly coarse, metatracheal parenchyma (figs. 37, 38). All the young wood specimens of sixteen species have diffuse and mostly narrow metatracheal parenchyma (figs. 12, 14).

C. decaisneana requires a special note (fig. 39). The old wood possesses diffuse and narrow metatracheal parenchyma. In addition, it presents nearly all the types of paratracheal parenchyma. Some of the vessels show scanty to abundant paratracheal parenchyma, while others demonstrate a complete jacket of parenchyma (vasicentric). Occasionally, the vasicentric parenchyma forms aliform wings which may link up to produce confluent parenchyma. Where apotracheal and paratracheal parenchyma types are both present (11), one cannot draw a line between metatracheal and banded paratracheal. As concentric laminae of wood parenchyma are present, it is necessary to describe this parenchyma as metatracheal to banded paratracheal.

The pitting between parenchyma cells in the old wood varies in size from less than 1 μ to 5.5 μ. The most frequent range of pit diameters is from 1.5 to 3.5 μ. Comparable pits in old wood tend to be smaller and range from less than 1 μ to 4.5 μ. The most frequent range is from 1.0 to 3.5 μ. The pits are many,



FIGS. 37–40.—Fig. 37, *C. fraseriana:* transverse section demonstrating coarse metatracheal and no diffuse parenchyma. ×85. Fig. 38, *C. fraseriana:* radial section showing broad metatracheal parenchyma in radial aspect. ×85. Fig. 39, *C. decaisneana:* transverse section to show various types of paratracheal parenchyma—scanty, abundant, vasicentric, aliform, and confluent. Notice transition from tracheids near vessels to fiber-tracheids at some distance from vessels. ×100. Fig. 40. *C. decaisneana:* radial section to show chambered parenchyma cells bearing crystals. ×100.

single, or clustered. No fusiform or septate parenchyma cells were observed, but chambered parenchyma cells with inclosed solitary, rhombohedral, supposedly calcium oxalate, crystals (figs. 5, 40) are abundant in the old wood and often in the young wood of all the species except *C. inophloia* and *C. junghuhniana*. Crystals are very rarely found in the typical wood parenchyma cells. The wood parenchyma cells are heavily lignified and only near the pith may cells conspicuously lacking in lignification be found. No sclerotic wood parenchyma cells were noted.

Two abnormalities may be described. If insect larvae form tunnels in the cambium, wound tissue, composed of irregularly isodiametric parenchyma cells, is formed and fills them. As growth of the cambium proceeds, and additional xylem is formed, the wound tissue or pith fleck is left as an island of parenchymatous tissue surrounded by the normal xylem. Pith flecks are fairly common in the wood of *Casuarina*. It should be mentioned that in *C. decaisneana* and *C. acutivalvis*, normally without aggregate rays in the old wood, what appear to be short-lived aggregate rays are formed at the region of injury. If the insect injury occurs in the already formed xylem, a winding tunnel remains, filled only with the debris of destroyed and broken tracheary cells.

ANATOMICAL DIFFERENCES AMONG BENTHAM'S SECTIONS

Most of the species classified by BENTHAM (**20**) were studied during this investigation and are listed under BENTHAM's sections in table 4. The species were compared in terms of all the anatomical characters used in the preceding description of the genus, but only the characters of value in the comparison appear in table 4.

The anatomical characters do not correspond entirely with the sections as set up by BENTHAM, although a few outstanding differences among the sections may be noted. *C. bicuspidata* of the section *Acanthopitys* (the only species of this section studied and of which there was only one specimen) differs from the other two sections in having a very high mean for the number of vessels per square millimeter and the lowest mean for vessel diameter. The sections *Leiopitys* and *Trachypitys* cannot be separated on these two characters; in fact, the only other constant character in the *Leiopitys* is the presence of scalariform perforation plates on some of the vessel elements. Unfortunately, this character also appears in two species of the *Trachypitys*.

If we add to the list those species which have been named and described since BENTHAM's *Flora australiensis*, Volume **6** (**20**), and then group the species on the basis of certain anatomical characters, more homogeneous sections are created. Those species with both scalariform and simple perforation plates may be placed in the *Leiopitys*. Those species with simple perforation plates and with a mean for the number of vessels per square millimeter below 100 may then be grouped in the *Trachypitys*, and those species with only simple perforation plates and with a mean for the number of vessels per square millimeter above 100 in the *Acanthopitys* (table 5, characters in bold-face type).

The *Leiopitys* as thus constituted still have quite variable characters, but any further modification seems impossible on an anatomical basis. BENTHAM had some difficulty in placing *C. decaisneana* in the *Trachypitys*. On the basis that *C. decaisne-*

ana and *C. torulosa* have scalariform perforation plates, they have been transferred from the *Trachypitys* to the *Leiopitys*. On the other hand, the *Trachypitys* have gained two species, while the *Acanthopitys*, as a group, are better substantiated by the addition of three new members. The *Trachypitys* and the *Acanthopitys* are much more homogeneous than the *Leiopitys* (table 5).

Leiopitys: Species means for the number of vessels per square millimeter in transverse section from about 8 to 75 (moderately few to very numerous but below 100); species means for the tangential diameter of vessels in transverse section from about 50 to 170 μ (moderately small to medium); scalariform perforation plates present on some or all of the vessel elements of a species (some species characterized by the presence of scalariform perforation plates may show in an occasional specimen a lack of this type of plate, but the majority of specimens will exhibit them), the remaining perforation plates simple; spiral thickenings in vessel elements variable, sporadic, and not always found on all specimens of some species, characteristically absent in some species, and characteristically present in others; type of metatracheal wood

TABLE 4

ANATOMICAL COMPARISON OF BENTHAM'S SECTIONS

Sections and species	Number of vessels per sq. mm. (Species means)	Diameter of vessels (Species means in μ)	Scalariform and simple perforation plates	Simple perforation plates only	Spiral thickenings in vessel elements	Metatracheal parenchyma, mostly narrow or coarse
Leiopitys:						
C. stricta	41	56	+*		s†	C§
C. glauca	44	63	+		s	N‖
C. equisetifolia	12	87	+		s	N
C. suberosa	75	66	+		+	N
C. cunninghamiana	36	75	+		s	N
C. distyla	65	55	+		−‡	C
C. fraseriana	17	95	+		−	C
Trachypitys:						
C. nana	67	52		+	+	N
C. humilis	60	46		+	+	C
C. torulosa	19	94	+		+	N
C. decaisneana	11	82	+		−	N
Acanthopitys:						
C. bicuspidata	304	43		+	+	N

* + = Spiral thickenings characteristically present on all or part of vessel elements.
† s = Spiral thickenings on vessel elements sporadic in certain wood specimens.
‡ − = Spiral thickenings characteristically absent.
§ C = Mostly coarse metatracheal parenchyma.
‖ N = Mostly narrow metatracheal parenchyma.

It is now possible to venture anatomical descriptions of the three sections, but it must be acknowledged that the changes in the grouping suggested herein are founded only on anatomical differences, since herbarium material was unavailable and could be not studied. The suggested revision of the generic sections, therefore, must be regarded as provisional until herbarium specimens and anatomical data can be harmonized.

parenchyma variable, mostly coarse in some species, mostly narrow in others.

Trachypitys: Species means for the number of vessels per square millimeter in transverse section from about 30 to 85 (numerous to very numerous but below 100); species means for the tangential diameter of vessels in transverse section from about 40 to 80 μ (very small to moderately small); simple perforation plates only; spiral thickenings in vessel elements characteristically present; metatracheal wood parenchyma narrow to coarse, mostly narrow, except in *C. humilis* in which it is mostly coarse; a fairly homogeneous group.

Acanthopitys: Species means for the number of vessels per square millimeter

TABLE 5

Sections and species	Indefinite growth rings present (+), generally completely lacking (−)	Imperforate elements: Percentage (quantity) of tracheids	Spiral thickenings	Vessels: Number of vessels per sq. mm., species means	Diameter of vessels in μ, species means	Walls predominantly thin (Th) or thick (Tk)	Scalariform and simple perforation plates (Sc) or simple plates only (Sm)	Number of bars in scalariform perforation plates (range)	Number of bars in scalariform plates (most frequent range)	Intervascular pitting transitional (T), opposite (O), alternate (A)	Spiral thickenings	Vascular rays (not including aggregate rays): Neither uniseriates nor multiseriates predominate (MU); uniseriates (U) or multiseriates (M) predominate	Aggregate vascular rays: "Cunninghamiana type" (Cu), "collina type" (Co), or "nearly compound" to compound (Cp)	Wood parenchyma distribution: Metatracheal parenchyma mostly narrow (N) or mostly coarse (C), paratracheal types also present (P)
Leiopitys:														
C. stricta	−‡	80–95	−	41	56	Th	Sc	4–15	12–15	O, A	S	MU	Cp	C
C. glauca	+	20–95	−	44	63	Th	Sc	2–20	8–15	A	S	MU	Co	N,C
*C. junghuhniana**	+	85–95	−	22	92	Th	Sc	12–20	13–14	A	S	MU	Cu	N,C
C. equiselifolia	+	5–20	−	12	87	Tk	Sc	6–21	8–17	A	S	M	.....	N,C
C. suberosa	+	10–90	+	75	66	Th	Sc	4–17	5–8	O, A	S	MU	Cu	N,C
C. cunninghamiana	+	60–90	−	36	75	Th	Sc	5–22	10–14	A	S	MU	Cu	N,C
C. distyla	+	30–90	−	65	55	Tk	Sc	2–5	2–5	A	S	MU	Co	N,C
C. torulosa†	+	70–100	S‖	19	94	Tk	Sc	3–19	8–11	A	S	U	Cu	N,C
C. decaisneana†	+	65–75	−	11	82	Th	Sc	3–8	4–7	A	S	MU	.....	N,P
*C. cambagei**	+	50–70	+	58	51	Th	Sc	2–10	5–7	A	S	M	.....	N,C
*C. dialsiana**	+	100	−	63	58	Th	Sc	4–10	7–8	O, A	S	MU	Cu	N,C
C. fraseriana	+	70–95	−	17	95	Th	Sc	4–14	10–13	O, A	S	MU	CuCp	N,C
*C. lepidophloia**	+	80–90	−	29	74	Th	Sc	8–17	9–10	A	S	MU	Co	N,C
*C. collina**	+	50–80	−	40	69	Tk	Sc	3–20	7–13	A	S	MU	Co	N,C
*C. montana**	+	85–95	−	17	64	Th	Sc	2–21	9–15	A	S	MU	Co	N,C
*C. deplanchеana**	+	85–95	−	8	109	Th	Sc	7–24	12–15	O, A	S	MU	Co	N,C
*C. poissoniana**	+	85–95	−	8	80	Th	Sc	8–21	14–16	A	S	MU	Co	N,C
*C. sumatrana**	−	60–90	−	8	162	Th	Sc	7–28	10–15	A	S	U	Co	N,C
*C. nodiflora**	−	20–70	−	8	122	Th	Sc	11–30	17–24	T, O, A	S	MU	Co	N
Trachypitys:														
C. nana	+	75–85	+	67	52	Tk	Sm			A	S	MU	Cp	N
C. humilis	+	30–40	+	60	46	Th	Sm			A	S	MU	Co	C
*C. inophloia**	+	90–100	+	82	53	Tk	Sm			A	S	U	Cp	N,C
*C. luehmanni**	+	85–95	S	33	73	Th	Sm			A	S	MU	Co	N
Acanthopitys:														
C. bicuspidata	+	60–70	+	304	43	Th	Sm			A	S	MU	Co	N
*C. acutivalvis**	+	80–90	+	262	34	Th	Sm			A	S	MU	.....	N,C
*C. baxteriana**	+	80–90	+	180	33	Th	Sm			A	S	MU	Cu	N,C
*C. muelleriana**	+	80–90	+	299	32	Th	Sm			A	S	MU	Cu	N

* Species given binomials since BENTHAM's publication.

† Species transferred from BENTHAM's *Trachypitys* to *Leiopitys*.

‡ − = Character characteristically absent in species.

§ + = Character characteristically present in species.

‖ S = Character generally absent but appears sporadically in certain specimens.

in transverse section from about 180 to 310 (very numerous but above 100); species means for the tangential diameter of vessels in transverse section smallest of the three groups, 30–45 μ (very small); simple perforation plates only; spiral thickenings in vessel elements characteristically present; metatracheal wood parenchyma narrow to coarse, predominantly narrow in all species; a homogeneous group.

POISSON (108) included BENTHAM's three groups in the *Cylindricae seu Cryptostomae* but placed *C. nodiflora*, *C. sumatrana*, and *C. deplancheana* (included in this investigation in the *Leiopitys*) in the *Tetragonae seu Gymnostomae*. To these three species he added several new species. None of these latter species described by POISSON is dealt with in this research.

It will be noted, however, in table 5 that *C. sumatrana*, *C. deplancheana*, and *C. poissoniana*—the latter described after POISSON's publication—are very similar in anatomical details. They all have fibers with extremely thick walls, heavily lignified ray cells, and aggregate rays very similar in structure. *C. nodiflora* also resembles these three species in all three characters. In addition, all four species have mostly narrow metatracheal parenchyma. Spiral thickenings in the vessel elements, however, are characteristically absent in *C. deplancheana* and *C. poissoniana*, always present in *C. nodiflora*, and are found in a few vessels of occasional specimens of *C. sumatrana*. On the whole, the four species closely resemble one another anatomically, and this resemblance would seem to support POISSON's division of the genus.

C. corniculata and *C. paludosa* have not been included in this discussion of the sections, as only young-wood specimens of these species have been examined.

DISTINGUISHING ANATOMICAL CHARACTERISTICS OF THE SPECIES

It is possible to identify the old wood of certain species or groups of species by the over-all aspects or combination of characteristics presented by microscopic examination, and table 5 attempts to depict the outstanding anatomical characters of the various species. The young-wood specimens are generally not considered in the tabulation, as the young wood in most cases reflects relatively little variation among the several species but usually fits into a typical pattern hardly capable of differentiation below the generic level.

The types of aggregate rays mentioned in table 5 must be described briefly. When the rays have very few fibrous elements, they should be considered as "nearly compound" (figs. 30, 31). In the "cunninghamiana type" of aggregate ray, most of the fibrous elements as seen in tangential section are vertical to nearly vertical, occur in fairly wide bands, and form an anastomosing framework which divides the ray into diamond-shaped or spindle-shaped segments. This arrangement is epsecially clear in *C. cunninghamiana* (fig. 35) and *C. fraseriana*. In the "collina type" the fibrous elements are oriented vertically and horizontally (directed both radially and tangentially) with the formation of a lattice-like framework which groups the ray cells into squares or rectangles as seen in tangential section (fig. 34).

C. corniculata and C. paludosa.—The anatomy of these species has not been referred to in table 5 because only young wood was available for study. The young wood of *C. corniculata* is composed of about 50% tracheids and 50% fiber-tracheids. Spiral thickenings are present in nearly all the fibrous elements. The mean for the number of vessels per square millimeter in transverse section is 275.

The vessels are angular to circular, and the walls are 1.5–6.5 μ thick. The mean for the vessel diameters is 27 μ. Scalariform perforation plates are absent, although wood immediately in contact with the pith was not examined. Intervascular pitting is alternate. Spiral thickenings are found in all the vessel elements. Fairly large aggregate rays, 0.1–0.5 mm. wide and 1–15 mm. long, are present, and they resemble those of *C. bicuspidata* and *C. collina*. The metatracheal parenchyma is mostly narrow.

Although the wood of this specimen cannot be considered old wood, neither is it first-formed wood. On this basis, it is possible to assign, tentatively, the species to a section. The absence of scalariform perforation plates, the high mean for the vessel number per square millimeter, the presence of spiral thickenings in the vessel elements, and the mostly narrow metatracheal parenchyma indicate that *C. corniculata* might be placed in the *Acanthopitys* close to *C. bicuspidata*.

The material of *C. paludosa* available for study was wood of a small twig, and little value can be placed on its characters. All the fibrous elements are tracheids, and their walls are thin to thick. Scalariform perforation plates are present, and the most frequent number of bars is 7–15. The intervascular pitting is transitional, opposite, and alternate, and the pit-pairs between the rays and the vessels are usually alternate but often opposite. Spiral thickenings occur in the fibrous elements and in the vessel elements. Small aggregate rays are present and are 0.15–0.4 mm. wide and 1.5–4.0 mm. long. The parenchyma is diffuse and narrow metatracheal. None of these characters is especially distinctive, as any one or all may appear in young-wood specimens of other species.

TAXONOMICAL NOTES ON CERTAIN SPECIES

C. stricta.—*C. quadrivalvis* Labill. Nov. Holl. Pl. ii 67 t. 218 was considered a synonym of *C. stricta* Ait. Hort. Kew. ed. I iii 320 (1789) by BENTHAM (**20**). No outstanding anatomical differences were found between the wood specimens representing these two entities, except that both of the specimens in which spiral thickenings were found on occasional vessel elements were labeled *C. stricta*. No spiral thickenings were found in any of the vessel elements of the specimens labeled *C. quadrivalvis*. Other variations have no correlation with the occurrence of spiral thickenings. As spiral thickenings are of sporadic occurrence in several species, it is felt that anatomical data support the reduction of *C. quadrivalvis* to a synonym of *C. stricta*.

C. equisetifolia.—*C. muricata* Roxb. Hort. Beng. 66; Fl. Ind. iii 519 (1832) is considered a synonym of *C. equisetifolia* Linn. Amoen. Acad. iv 143; Forst. Char. Gen. 103 f .(1776) (**45**). One wood specimen of *C. muricata* (Y9941) was examined and was found to differ chiefly in that only simple perforation plates were present and that spiral thickenings occurred in a few vessel elements. As one other specimen of wood (H3402) labeled *C. equisetifolia*, however, also possessed these characters, no conclusion can safely be stated in regard to the synonymy of *C. muricata*.

C. suberosa.—*C. leptoclada* Miq. Rev. Crit. Casuar. 41 t. 4 (1848) was reduced to a synonym of *C. suberosa* Otto and Dietr. Allg. Gartenz. 155 (1841) by BENTHAM (**20**). Since three specimens lack spiral thickenings (fig. 22) which are prominent and constant in the other ten (fig. 23), it might prove profitable to examine more thoroughly the taxonomic characters of this species. If it were found

that there are two separate species, it would then be interesting to check for the presence or absence of spiral thickenings in the vessels.

C. muelleriana.—Although only one old-wood specimen of *C. muelleriana* was examined, certain tentative conclusions may be mentioned in regard to the taxonomy of the species. *C. muelleriana* Miq. in Neder. Kruidk. Arch. iv: 99 (1859) was reduced to a synonym of *C. distyla* Vent. Jard. Cels. t. 62 (1803) by POISSON (**108**). MACKLIN returned this binomial to species rank in 1927 (**89**). The anatomical differences between the two species render strong support to their separation by MACKLIN.

C. distyla is considered in this investigation as one of the *Leiopitys*, whereas *C. muelleriana* is considered as a member of the *Acanthopitys*. The species mean for the vessel number per square millimeter is 65 in *C. distyla*, while it is 299 in *C. muelleriana*. Most of the vessel walls in *C. distyla* are 6–8 μ thick, whereas in *C. muelleriana* they are mostly 3–5 μ and only rarely thicker. The species mean for the vessel diameters is 55 μ in *C. distyla* and 32 μ in *C. muelleriana*. The vessel elements in the old wood of *C. distyla* may or may not have scalariform perforation plates and do not have spiral thickenings. In *C. muelleriana* the vessel elements have spiral thickenings and only simple perforation plates. The aggregate rays in the wood of *C. distyla* resemble those of *C. collina*, and the metatracheal parenchyma is frequently coarse (6–7 cells wide); while in *C. muelleriana*, the aggregate rays resemble those of *C. cunninghamiana* and the metatracheal parenchyma is mostly narrow (1–2 cells wide), although very rarely 3–4 cells wide. A further difference is found in the distribution of the species. *C. distyla* Vent., sen. str. Macklin is found in Tasmania while *C. muelleriana* Miq. is found in South Australia (**89**).

C. baxteriana.—Anatomical data seem to support MACKLIN's (**89**) separation of *C. baxteriana* from *C. distyla*. Again, however, the following facts must be considered with some reservation, as one old-wood specimen of *C. baxteriana* was examined. *C. baxteriana* Miq. Rev. Crit. Casuar. 57 t. 3 (1848) was reduced to a synonym of *C. distyla* Vent. Jard. Cels. t. 62 (1803) by POISSON in 1876 (**108**). The species mean for the vessel number per square millimeter is 65 in *C. distyla* and 180 in *C. baxteriana*. The species mean for the vessel diameters in *C. distyla* is 55 μ, but it is 33 μ in *C. baxteriana*. The vessel elements have scalariform and simple plates and may or may not have spiral thickenings in *C. distyla*, but in *C. baxteriana* the vessel elements have spiral thickenings and only simple perforation plates. The wood of *C. distyla* has aggregate rays and mostly coarse metatracheal parenchyma (often 6–7 cells wide), but *C. baxteriana* has no aggregate rays in the old wood and mostly narrow metatracheal parenchyma (never over four cells wide).

The anatomy of the secondary phloem

The secondary phloem contains phloem parenchyma cells, ray cells, phloem fibers, stone cells, sieve-tube elements, and companion cells. In transverse section the thin-walled parenchyma, ray cells, sieve tubes, companion cells, and phloem fibers form a continuous network inclosing areas of thick-walled, sclerenchymatous parenchyma elements.

The vascular rays, both uniseriate and multiseriate, broaden in width in the phloem. The tangential diameter of the ray cells increases progressively in the

cells farther and farther from the cambium. To a large extent, the ray cells and, to a lesser extent, the phloem parenchyma cells form the ground tissue of the phloem.

Many of these short parenchymatous ray cells become converted into very thick-walled cells inclosing rhombohedral crystals or enlarge greatly to form stone-cells with ramiform pits. These latter cells may have their long axes directed radially, tangentially, or vertically. Indeed, the most striking feature of the phloem in *Casuarina* is the large amount of sclerification.

In radial or tangential sections it may be seen again that the sieve-tube elements, companion cells, phloem fibers, and phloem parenchyma—the elongate elements—form an anastomosing system, while the thin-walled and thick-walled ray cells form a compact ground tissue.

The sieve tubes in the bark from old trees are composed of sieve-tube elements of the second type (**67**). They are of moderate length (approximately 165–245 μ) and have oblique end walls, each end wall extending about one-eighth of the total length of an element. On each end wall there are five to ten sieve plates. The side walls of the sieve tubes bear the lattice-work of vestigial sieve plates with no visible pores. The sieve-tube elements of the phloem of 1- to 2-year old branches tend to be longer and more narrow in diameter. The side walls of these elements bear circular to oval vestigial plates which usually lack pores. Occasionally, these sieve plates may have indistinct pores; and, therefore, it is doubtful if they are functional.

The companion cells are difficult to discern, but apparently there is only one associated with each sieve-tube element. The companion cell is usually about two-thirds the length of its sieve-tube element.

There seems to be little crushing or obliteration of the phloem as it is forced outward by the formation of new phloem within. In young branches, 1–2 years old, the phloem contains very few sclerenchymatous cells and is surrounded by the cortex which is composed of typical, isodiametric, thin-walled cells. The phellogen arises originally in the cortex, but successive formations soon develop in the phloem. The phellem consists of dead, thin-walled, nearly isodiametric cells, which are slightly longer tangentially. The cells are filled with a light- to dark-brown material which probably is largely tannin.

Discussion

The relation of the Casuarinaceae to angiosperm phylogeny

The systems of classification that have been proposed since the time of Linnaeus are numerous and varied, but there emerges from the study of them the conclusion that there are two chief theories for the origin, classification, and phylogeny of the angiosperms. Each theory is constructed on its fundamental conception of the primitive angiosperm type.

The first theory, in essence, considers that the angiosperms were derived from coniferous- or gnetalian-like ancestors and that the extant gymnosperms represent a stage of development through which the angiosperms must have passed in their evolution. The most primitive angiosperms, then, should possess characters that are not greatly different from those of the living Coniferales and Gnetales. The habit should be arborescent or shrubby. The flowers should be apetalous, anemophilous, unisexual, of few parts, and grouped in conelike in-

florescences or catkins. Evolution must have proceeded, therefore, by the transformation of bisexual, anemophilous inflorescences into entomophilous flowers: by the formation of a perianth from the large outer bracts (or development *de novo*); by the telescoping of the inflorescence axis; by fusion of the bracteoles surrounding the ovules to form carpels; and by the loss of the remaining bracteoles between flowers. Accordingly, the so-called Amentiferae provide the necessary primitive angiospermous characters, and the Gnetales are usually cited as the transitional gymnospermous forms. The systems of ENDLICHER (**48**), EICHLER (**47**), WETTSTEIN (**140**), and of ENGLER and his school (**49, 50, 51, 79, 115**) are similar in following this theory. The Apetalae are considered primitive, and the Monochlamydeae are thought of as transitional to the derived, polypetalous Magnoliales and allied groups.

The system of ENGLER, published in the first edition of *Die natürlichen Pflanzenfamilien*, first appeared without explanation. In two articles published in a supplement to Volumes **2–4** (**50**), however, ENGLER offered a synopsis of his classification of the seed plants or Siphonogama, followed by an explanation of his system. He stated that the most important character determining the arrangement of his alliances is the evolution of the flower—specifically, the development of a perianth. In the dicotyledons he considered the absence of a perianth (achlamydie) primitive, and the normal course of evolution the gradual appearance of a perianth, first as slightly modified bracts, then as a single perianth jacket (homoiochlamydie), and finally as a double and differentiated perianth (heterochlamydie). The evolution of the fruit and ovule stands next in importance.

For reasons which he did not fully explain, he considered the presence of numerous fertilizable embryo sacs, developing from numerous macrospores, and chalazogamy as outstanding primitive characters; and, chiefly on the basis of these two characters, placed the Verticillatae (Casuarinales) first among the achlamydeous orders, followed by the Piperales, Myricales, and Leitneriales. The Juglandales and Fagales stand first among the monochlamydeous orders, since they possess primitive bracteate perianths and, again, chalazogamy.

When the polypetalous orders were finally reached, beginning with the Ranales, he invoked hypogyny, apocarpy, and the spiral arrangement of parts as primitive characters.

In Volume **14***a*, published in 1926 (**51**), the question of angiosperm origin was discussed in great detail, and dicta or *Prinzipien* were presented. The latter were repeated in the eleventh edition of the *Syllabus der Pflanzenfamilien* (**49**). ENGLER stated that the angiosperms have been derived from gnetalian-like ancestors, and he set up a hypothetical group, the Protangiospermae, to stand between ARBER and PARKIN's Hemiangiospermae (**1**) and the modern angiosperms.

It is pertinent to describe a few of the transitional features presented by this hypothetical group. The Gnetales have a saclike perigonium, but the Protangiospermae displayed an incipient perianth to account for the appearance of a perianth in the angiosperms. Although plants in the Gnetales are generally dioecious, occasional bisporangiate strobili occur (**33**). The Protangiospermae bore bisexual flowers (probably a result of the supposed tendency toward hermaphroditism initiated in the Gnetales) to account for the prevalent hermaphroditism in the

angiosperms. Yet, ENGLER's primitive angiospermous orders were dioecious or monoecious. The exothecium of the gnetalian stamen became, without sufficient explanation, an endothecium in the hypothetical group to provide for the origin of the endothecium of the angiospermous stamen. The presence of numerous macrospores in the Casuarinaceae was considered a primitive character, even though the majority of extant gymnosperms have lost this condition. To support the theory, and to account for the many macrospores of the Casuarinaceae, the hypothetical Protangiospermae were supposed to have revived this pteridophytic character.

ENGLER explained the conception of chalazogamy as a primitive character much as did WETTSTEIN (**140**). In the higher gymnosperms the pollen tube grew through the nucellus to the egg cell before fertilization. The development of the ovarian envelope in the simplest angiosperms hindered the access of the pollen tube to the nucellus. The pollen tube, however, retained its ability of growth through tissue and grew, therefore, through the style, the funiculus of the ovule, and the chalaza. This is seen in the primitive orders of ENGLER and of WETTSTEIN (Casuarinales, Fagales, and Juglandales). Porogamy was derived from chalazogamy through the intermediate stage of mesogamy. In mesogamy the pollen tube may enter through the micropyle or in its vicinity, but its path is from the style either to the base of the ovule and then up through one of the integuments as in *Alchemilla arvensis* (**87**) and *Cucurbita pepo* (**97**) or from the funiculus horizontally across the top of the integument as in certain species of *Ulmus* (**103**) and in *Cannabis sativa* (**143**). This lengthy account seems justified, as the writer is not aware of any adequate discussion in the English language of the morphological basis for ENGLER and PRANTL's system.

WETTSTEIN's classification of the dicotyledons (**140**) is similar to that of ENGLER and PRANTL. WETTSTEIN, however, went into greater details of morphology than ENGLER, although the general hypothesis was the same. The chief contribution of WETTSTEIN was the familiar theory for the derivation of the petaliferous angiosperm flower from the compound inflorescence of *Ephedra*. The catkin of *Casuarina* was interpreted as intermediate in this evolution. It is assumed that WETTSTEIN employed *Ephedra* as a living example of a previous stage through which the angiosperms passed.

The second theory considers the primitive angiosperms to be those with flowers that have a perianth and many free or nonfused parts arranged spirally on an elongated axis. The flowers should be hermaphroditic, entomophilous, and hypogynous. Characters such as achlamydy, oligomery, sympetally, synsepally, zygomorphy, epigyny, syncarpy, anemophily, etc., are derived. It is often assumed by this hypothesis that the angiosperms were derived from some gymnospermous ancestor which possessed loosely arranged, many-parted strobili with megasporophylls above, microsporophylls below, and, still lower, sterile leaves which may have become perianth parts. Certain fossil Cycadophytae rather than fossil Coniferophytae are considered to demonstrate incipient angiospermous characters, and the Magnoliales are adjudged the most primitive extant angiosperms. This theory may be termed the ranalian theory.

With BENTHAM and HOOKER (**21**) the ranalian theory made its first appearance as a system of classification. Beginning in

1897 BESSEY (**22, 23**) published a series of papers in which there was outlined for the first time in the English language a series of dicta describing the lines of angiospermous evolution and a system of classification based upon these principles. It will suffice to say that BESSEY's system probably is the one which embodies most completely the concepts of the ranalian theory. The system placed the Ranales as the most primitive group and considered such derived orders as the Rosales, Caryophyllales, and Primulales as secondary plexuses for the derivation of higher orders. The monocotyledons were derived from the dicotyledons through the Alismales. BESSEY (**22**) included the Casuarinaceae in the Sapindales but later placed the family in the Rosales as leafless representatives of the Hamamelidaceae.

HALLIER (**66**) published a system very similar to that of BESSEY. The amentiferous orders were relegated to a position near the end of the scheme. The Hamamelidaceae appeared in the Amentiflorae, and the Myricaceae, Salicaceae, Juglandaceae, and Betulaceae were considered as derived from them. The Casuarinaceae were reduced to a tribe of the Betulaceae. ARBER and PARKIN proposed a theory for the origin of the angiosperms and created several purely hypothetical forms to account for the transition from the Bennettitales or bennettitalian-like ancestors to the known late Cretaceous and Tertiary angiosperms. This hypothesis, common knowledge to phylogenists today, strongly supported the ranalian theory. The Gnetales were considered by ARBER and PARKIN (**2**) as the last members of a race (reduced and arrested Proanthostrobilatae) which arose from the same hemiangiospermous stock as the angiosperms. HUTCHINSON (**69, 70**) worked out a scheme of phylogeny which, again, is essentially similar to that of BESSEY. As far as possible, the Monochlamydeae were distributed among the Polypetalae as reduced and recent forms. The Casuarinales were still placed near the Urticales, Fagales, Myricales, Leitneriales, Garryales, and Salicales and were considered to be reduced from the Hamamelidales. The latter order was derived from the Rosales and considered as transitional to the amentiferous orders.

A few additional authors should be mentioned. TREUB (**134**), on the basis of chalazogamy, separated the Casuarinaceae from the other dicotyledons and formed the Chalazogames, a subdivision equivalent to the Porogames, containing the remaining dicotyledons and monocotyledons. RENDLE (**114**) gave a succinct translation of TREUB's epochal paper and criticized him for separating the Casuarinaceae from the other angiosperms on a single character.

VUILLEMIN (**136**) presented probably the most fantastic idea for the relationship of *Casuarina*. He believed *Casuarina* to represent an arrested form transitional between the Hepaticae and the seed plants. He thought the tracheids among the macrospores to be the vestiges of the columnar axis of the bryophytic sporangium. On the basis of supposed similarities between the outer vascular system of the very young *Casuarina* stem and the vascular system of *Equisetum*, and the similarity of the jointed stems and reduced leaves in both genera, CORDEMOY (**39**) derived *Casuarina* from the aborescent Calamodendraceae (Calamitaceae).

PHYLOGENY OF THE CASUARINACEAE AS INDICATED BY ANATOMICAL EVIDENCE

This discussion is an attempt to evaluate the anatomical characters of the

Casuarinaceae in the light of the phylogenetic dicta listed in the introduction; for each character of phylogenetic value the existing condition in the family is pointed out as either primitive or specialized.

The wood of the genus presents only one primitive character of importance, the solitary distribution of the vessels. There is an imposing array of characters, however, which signifies not primitiveness but a moderate or intermediate phase of evolutionary advancement. These characters are: the presence of fiber-tracheids as well as tracheids but the lack of libriform wood fibers; the length of fibrous elements, very short to medium-sized; spiral thickenings in the fibrous elements and vessel elements of several species but absent in others; the predominance of circular thin-walled vessel elements but with some angular, thin-walled and some angular to circular thick-walled vessel elements; vessels very small to medium-sized; the predominance of vessel elements with simple perforation plates, although in many species a low proportion of the elements have scalariform plates with narrow apertures and bars with complete borders; the prevalence of the intermediate number of bars composing the scalariform perforation plates; the occurrence in most species of at least a few vessel elements with transverse end walls and the predominance of vessel elements with end walls between 40° and 80°; the very short to moderately long vessel elements; the heterogeneous type IIB rays and the tendency toward the elimination of either the uniseriates or the multiseriates in certain species; the common occurrence of aggregate rays and the trend toward their dissection and loss; and, finally, the constant presence of metatracheal parenchyma, the tendency toward the elimination of the diffuse parenchyma, and the development of paratracheal types. There is one character, also, that may be cited as highly specialized—the overwhelming prevalence of alternate intervascular pitting. The presence of only simple perforation plates on the vessel elements of the *Trachypitys* and *Acanthopitys* might also be added as a highly specialized condition.

Certain limited conclusions may be drawn on the basis of the anatomical evidence. As the secondary wood of *Casuarina* exhibits so few anatomical characters which have been proved as primitive and so many characters which have been proved as specialized or advanced, it is suggested that the Casuarinaceae are a specialized family of the angiosperms and not a primitive group. The evidence also indicates that, although *Casuarina* presents many specialized characters, the degree of specialization is only moderately advanced. Further than this it is not possible to go until evidence from other sources is considered.

A CORRELATION OF EVIDENCE FROM ANATOMY AND FLORAL MORPHOLOGY

BESSEY (**22, 23,**), HUTCHINSON (**69**), and HALLIER (**66**) derived the Casuarinaceae from the Hamamelidaceae, chiefly on the basis of floral morphology. HALLIER and HUTCHINSON, in turn, derived the Hamamelidaceae from the Magnoliales. BESSEY derived the Rosales (including the Hamamelidaceae) from the Magnoliales. Even WETTSTEIN (**140**) and ENGLER and PRANTL (**50, 51**) saw a relationship between the Rosales and the Hamamelidaceae. WETTSTEIN, however, placed the Hamamelidaceae before the Polycarpicae, and the latter group was considered as derived. According to most adherents of the ranalian theory, therefore, the Hamamelidaceae are considered

as derived from the Magnoliales, or from an ancestral group similar to the extant Magnoliales, by reduction. Reduction in parts seems to be one of the chief lines of floral evolution in the derivation of angiospermous groups from the Magnoliales, and the Rosales, including the Hamamelidaceae, give abundant evidence of this reduction tendency.

If the outstanding floral characteristics of the Casuarinaceae are enumerated, the resemblance between the Casuarinaceae and the Hamamelidaceae is startling. It may be easily conceived that the trends toward reduction initiated in the Hamamelidaceae have progressed to extremes producing the conditions found in the Casuarinaceae: plants monoecious or dioecious; the male flowers in spicate inflorescences, the female in capitate inflorescences; male flowers with two (or one) small perianth leaves, female flowers without perianth; stamens reduced to one; ovary superior, composed of two carpels, one-celled at maturity but showing suppression of one locule in ontogeny, usually only one maturing; and seed solitary, with a straight embryo and no endosperm. According to the supporters of the ranalian theory, the Casuarinaceae, on the basis of floral characteristics, could have been derived from the Hamamelidaceae by further reduction. A comparison of the above floral characteristics with those of the Hamamelidaceae clarifies this hypothesis.

Two additional authors, among others, have supported the ranalian theory. ROBERTSON (**119**) believed that syncarpy and polyspermy were entomophilous characters. He found it difficult to imagine syncarpy arising in anemophilous groups; rather, he considered that the syncarpy of anemophilous orders was carried over from entomophilous groups. Also, polyspermy correlated with syncarpy and dependent on entomophily could hardly have arisen in anemophilous groups where it was difficult for the female flowers to obtain enough pollen for a single ovule. PARKIN (**107**), in a detailed examination of inflorescence types, clearly stated that the solitary terminal flower was primitive and that the inflorescence (including the catkin) was derived.

The systems founded upon the ranalian hypothesis (**1, 21, 22, 23, 69, 70, 92**) and the various lines of supporting evidence based upon floral morphology, therefore, lend support to the hypothesis that the Casuarinaceae are an advanced, yet reduced, and derived group rather than a primitive family (**47, 48, 49, 50, 51, 140**). There is also a general agreement, among the authors supporting the ranalian hypothesis, that the Casuarinaceae have been derived from the Hamamelidaceae. At present, no evidence can be offered by floral anatomy, as far as the Casuarinaceae are concerned.

It has been stated previously that the Casuarinaceae on anatomical evidence cannot be considered a primitive (or the primitive) group of the Angiospermae. This conclusion is founded on phylogenetic dicta of anatomy which are in turn based on evidence assembled without reference to preconceived systems of phylogeny. The anatomical evidence coupled with the evidence of the derived nature of the flower makes it impossible to consider the Casuarinaceae as a primitive group and requires that the family be derived, if possible, from some other group of the angiosperms. Floral structure indicates that the Casuarinaceae have been derived from the Hamamelidaceae, and floral morphology also supports the view that the Hamamelidaceae are in turn reduced Magnoliales. It is im-

portant, now, to see if the anatomical evidence will substantiate these claims.

The Magnoliales have many primitive anatomical characters to recommend their position as the most primitive group of the Angiospermae (90). TIPPO (129) summed up the anatomical evidence for this order in the following manner:

> The Magnoliales exhibit a considerable range of anatomical specialization, yet the more primitive groups (anatomically) may be characterized as follows: Some of the members have no vessels. Many of the forms have exclusively scalariform perforation plates. The perforations are completely bordered, or bordered to the middle, or bordered at the ends. Many species have many bars on the perforation plates; often the perforations are narrow. The vessel elements are characterized by great length and small diameter. The vessels are usually angular and thin-walled. The vessel end walls are very oblique. Intervascular pitting is scalariform in many. The woods are diffuse-porous. Most of the vessels are solitary. The fibrous tracheary elements in many species are tracheids exclusively. The fibrous tracheary elements are very long. Many of the forms have heterogeneous I rays. Some of the species have diffuse parenchyma. Almost all forms are arboreal in habit. On the basis of anatomy, therefore, there is much to recommend the Magnoliales as a very primitive order among the Dicotyledons.

The Hamamelidaceae, in turn, present many primitive anatomical features and are, anatomically, scarcely higher than the Magnoliales. The pronounced reduction of the flowers indicates that they have become specialized, yet their anatomical characters signify a close relationship between the two groups. TIPPO (129) derived the Hamamelidaceae from the Magnoliales by use of the "anatomical method." The outstanding anatomical features of the Hamamelidaceae (129) are:

> All but one species have tracheids exclusively; the vessels are mostly solitary; the woods are diffuse-porous; the vessels are angular, thin-walled, and extremely small; the perforation plates are exclusively scalariform; the end walls of the vessel members are very oblique; the intervascular pitting is scalariform, transitional, and opposite; the vessel elements are very long; the rays are usually heterogeneous I, or IIA; and the xylem parenchyma is diffuse in most species.

The Casuarinaceae, on the other hand, as indicated previously, present anatomical characters that are moderately specialized and which may be considered to denote an evolutionary level above but not far above the Hammamelidaceae. The occurrence of tracheids and fiber-tracheids but not libriform wood fibers; the presence of circular, thin-walled as well as circular thick-walled, moderately small to medium-sized vessels; the presence of simple as well as scalariform perforation plates, yet only simple in some species; the occurrence of some vessels with transverse end walls; the predominance of alternate intervascular pitting; the heterogeneous IIB rays; and the constant appearance of metatracheal parenchyma but also of diffuse parenchyma: all these characters denote that the Casuarinaceae have evolved beyond, yet not far beyond, the Hamamelidaceae. On the contrary, however, the universally solitary distribution of the vessels; the diffuse-porosity with only a slight tendency to ring-porosity; the occurrence of angular thin-walled vessels and occasionally angular thick-walled vessels in some species (also almost universally angular and thin-walled in young wood); the occurrence of scalariform plates in many species (and in the young wood of nearly all species); the predominance of vessel elements with oblique end walls; the occurrence of transitional intervascular pitting in one species and opposite in several; the retention of the diffuse parenchyma in most species: all these characters suggest the possibility of a close

relationship between the Casuarinaceae and the Hamamelidaceae.

It may be said, then, that the anatomical evidence obtained from the investigation of the Casuarinaceae supports, or at least is not inconsistent with, the derivation of the Casuarinaceae from the Hamamelidaceae. In this matter the author is in agreement with TIPPO (**129**), who, in his survey of the amentiferous orders, derived the Casuarinaceae from the Hamamelidaceae. The author also agrees with TIPPO that the Casuarinaceae are at an anatomical level of evolution higher than that of the Betulaceae and lower than the Fagaceae.

EVIDENCE FROM EMBRYOLOGY AND GAMETOPHYTIC MORPHOLOGY

SWAMY (**127**) has surveyed the literature on embryological and gametophytic morphology of *Casuarina*, and the present author has drawn heavily on SWAMY's manuscript as a basic source of information on this subject. TREUB (**134**) was the first to publish on the embryology of *Casuarina*. He reported such oddities as the formation of sporogenous tissue by proliferation of other sporogenous cells and the formation of tracheids in the sporogenous tissue in the region of the chalaza, the massive sporogenous tissue, the simultaneous development of many fertilizable embryo sacs, the absence of antipodals, the formation of fifty endosperm nuclei before fertilization, the chalazogamic path of the pollen tube, and other aberrations. Although no one has repeated TREUB's work on *C. suberosa*, the investigation of other species (**59, 81, 127**) casts serious doubt on TREUB's report of the absence of antipodals and the formation of endosperm nuclei before fertilization.

The interest in these peculiar features of the gametophyte lies in the fact that many authors conclude that, because certain characters of a group differ from those in the majority of the angiosperms, the group in question must be primitive. The papers of TREUB, JUEL, FRYE, and SWAMY yield the following pertinent "differences": the formation of massive sporogenous tissue, the frequent division of each spore mother cell producing numerous macrospores, the maturation of a number of macrospores into fertilizable embryo sacs, and the phenomenon of chalazogamy.

Owing to the fact that nearly all these aberrant characters have been discovered in a number of plants in various unrelated groups throughout the Angiospermae, it is now thought that these atypical characters are sporadic peculiarities which have little if any phylogenetic significance. It is particularly important (**121**) to note that the embryo-sac development in *Casuarina* is of the normal type. In addition, the embryo is straight and develops from a linear series of cells as in most angiosperms. Massive sporogenous tissue occurs in the Rosaceae and the Malvaceae (**120**) as well as in the Casuarinaceae. Numerous macrospores are now known to occur also in the Ranunculaceae, Rosaceae, Onagraceae, Compositae, Fagaceae, and Umbelliferae (**120**). It is particularly significant that many macrospores have been discovered in *Hamamelis* (**122**), and this fact gives added support to the thesis that *Casuarina* is derived from the Hamamelidaceae. As far as this author is aware, no other cases of numerous fertilizable embryo sacs have been reported for the angiosperms.

True chalazogamy is now known to occur in the Casuarinaceae (**59, 127, 134**), the Betulaceae (**99, 100, 104**), the Corylaceae (**18, 19, 55, 101, 105**), and the Juglandaceae (**24, 82, 98, 102, 106, 142**).

Mesogamy occurs in certain members of the Ulmaceae (103), the Cannabinaceae (143), the Cucurbitaceae (87), and in *Alchemilla arvensis* of the Rosaceae (97). It seems more reasonable to interpret chalazogamy as having arisen in certain members of the Amentiferae through the transitional stages of mesogamy seen in other groups (40) than to suppose with ENGLER and PRANTL and with WETTSTEIN that porogamy has arisen from chalazogamy through mesogamy.

In summary, the author is of the opinion that these peculiarities cannot be given as much weight in our phylogenetic schemes as the numerous anatomical characters which have been demonstrated repeatedly to be of phylogenetic value. Nor can these sporadic aberrations be considered of much phgeylonetic value in the presence of far more constant characters in the majority of angiosperms, such as the nonmotile sperms, the presence of closed carpels, double fertilization, and the eight-celled embryo sac. It is concluded, then, that these unusual gametophytic characters of the Casuarinaceae do not invalidate the contention that the family is not primitive or invalidate the conclusion that the family is moderately specialized and probably derived from a more primitive group as the Hamamelidaceae.

MISCELLANEOUS LINES OF EVIDENCE

SECONDARY PHLOEM.—Two important conclusions may be drawn from the investigation of the secondary phloem of *Casuarina:* the secondary phloem is highly specialized in its extreme sclerification of the fibers, stone cells, and thick-walled ray cells; yet, the diagnostically important sieve-tube element being of the second type, has advanced only moderately. In other words, the phloem has reached an evolutionary level equal to that of the xylem. This evidence again indicates that *Casuarina* is not primitive but moderately advanced or specialized.

NODAL ANATOMY.—SINNOTT (123) has shown that there are three types of nodal structure in the angiosperms. The trilacunar condition was considered primitive and the unilacunar and multilacunar states derived. The Casuarinaceae (123) have a single trace entering each leaf. One gap is formed by each trace. The Hamamelidaceae, on the other hand, demonstrate the more primitive trilacunar condition. This evidence, in conjunction with other evidence, supports previously stated conclusions.

CYTOLOGY.—The basic chromosome number of *Casuarina* is probably twelve (60, 61, 127), although various numbers have been reported. The Hamamelidaceae demonstrate most commonly numbers of twelve and fifteen (60, 61, 62, 132, 133, 137). It is not inconsistent, in view of the other evidence favoring the derivation of the Casuarinaceae, to consider that the chromosome number of twelve found in members of the Casuarinaceae substantiates a relationship to the Hamamelidaceae (137).

PALEOBOTANY.—Three species of *Casuarina* have been reported from the Tertiary of Europe (54), but grave doubts have been cast on the identification of these species (144). Two fossil wood specimens of *Casuaroxylon* of unknown locality or horizon (80) and one extremely doubtful vegetative specimen from the Lower Cretaceous of America (138) have been described. Questionable species from the Tertiary and Cretaceous deposits of Australia, New Zealand, and Tasmania have been mentioned (41, 85). At the present it is only possible to say that the scarcity, or lack, of authenticated fossil specimens of the Casuarinaceae in no way negates but rather

favors the conclusions that the family is a moderately advanced one and probably derived from the Hamamelidaceae.

POLLEN MORPHOLOGY.—The conclusion that the Casuarinaceae are not primitive is further supported by the type of pollen grain found in this family. The grains are not of the most primitive monocolpate type (**141**) found in the Magnoliaceae nor are they of the more advanced tricolpate type found in the Hamamelidaceae. The pollen grains of *Casuarina equisetifolia* are acolpate, with three equatorial germinal pores (**52**); they are, therefore, probably derived from the tricolpate type.

ECOLOGY.—The majority of the Casuarinaceae thrive either along the coast in brackish areas or in sand dunes or on dry, sandy, upland savannas. They grow, then, in soils that are either physiologically or climatically arid. It is felt that this corroborates the view that the supposed simple characters are the result of reduction rather than primitiveness. The superficial resemblance among *Casuarina*, *Pinus*, *Equisetum*, and *Ephedra* is explainable on the grounds that they are all xerophytes or near-xerophytes. In other words, the resemblance is the result of convergent evolution, just as the unrelated spurges, cacti, aloes, and agaves, which grow in the same habitats, are strikingly similar in appearance.

Summary

1. The chief purposes of this investigation have been to contribute toward a better understanding of the evolutionary status of the Casuarinaceae; to present a thorough anatomical description of the family; and to tabulate, at least provisionally, the outstanding characteristics of twenty-nine species.

2. The anatomy of the secondary xylem of the old wood (wood at some distance from the pith) may be summarized as follows: Growth rings are poorly defined or absent; both tracheids and fiber-tracheids are found in all but one species, in which only tracheids occur; tracheids generally predominate, and the larger, thinner-walled tracheids are vasicentric; the fiber-tracheids often have very thick walls; the fibrous elements are extremely short to moderately long, mostly short to medium-sized; spiral thickenings occur in the fibrous elements of six species. The range of species means for the number of vessels per square millimeter is from moderately few to very numerous; vessel distribution is universally solitary; diffuse-porosity is characteristic, although there is a very slight tendency toward ring-porosity; the majority of species have vessel elements which are thin-walled, circular in outline, although thick-walled, circular to somewhat angular vessels occur in seven species. Vessel diameters range from very small to moderately large (genus mean is 63 μ, moderately small), mostly from very small to medium-sized. The old wood of eight species has vessel elements with only simple perforation plates; the remaining species have scalariform and simple perforation plates. Among the species with scalariform plates, the majority have plates with the intermediate number of bars; the bars of the scalariform plates are completely bordered, and the openings between the bars are predominantly narrow. The majority of the species have vessel elements with sloping end walls, most frequently from 40° to 80°; transverse end walls are not uncommon in some species, however. Intervascular and ray-vessel pitting is largely alternate. The range of vessel-element lengths falls in the very short to moderately long classes; the genus mean is 439 μ (medium-sized). Spiral thickenings occur

characteristically in the vessel elements of thirteen species and sporadically in five species; the remaining species have vessel elements with generally no spiral thickenings. The vascular rays are heterogeneous IIB. Aggregate rays occur in the old wood of twenty-three species but are absent in four species. Metatracheal parenchyma occurs in all species, diffuse in nearly all, and paratracheal types in one.

3. The taxonomic divisions of the genus by BENTHAM and by POISSON are considered on an anatomical basis. In general, the anatomical evidence supports the respective divisions of the genus, but it is suggested that *C. torulosa* and *C. decaisneana* might be better placed in *Leiopitys*. Anatomical characters are used to assign to sections those species given binomials since the time of BENTHAM and of POISSON. Tentative suggestions are offered, based on anatomical data, for a better understanding of certain species. A survey of the present taxonomical status of the genus revealed that a taxonomic monograph would be extremely valuable.

4. The phylogenetic conclusions based on anatomical evidence are as follows: the Casuarinaceae are a specialized family of the Angiospermae and are not a primitive group. The evidence also indicates that, although *Casuarina* presents many specialized characters, it is moderately rather than highly specialized.

5. Floral morphology indicates that the Casuarinaceae have been derived from Hamamelidaceae-like ancestors. The lines of floral reduction seen in the Hamamelidaceae seem to have been carried to extremes in the Casuarinaceae. A study of the correlation between anatomy and flower structure of the Casuarinaceae precludes the retention of the family as a primitive (or the primitive group) of the Angiospermae. In addition, the anatomical evidence presented supports, or at least is not inconsistent with, the derivation of the Casuarinaceae from hamamelidaceous ancestors as suggested by floral morphology.

6. A tabulation of the distinguishing characteristics of the species is offered. In most cases, combinations of anatomical characters distinguish the species or groups of species.

7. The aggregate ray in *Casuarina* is considered to arise in ontogeny, and to have arisen in phylogeny, by the aggregation of uniseriate and multiseriate rays. The aggregate and compound rays undergo dissection to some extent in the later development of the xylem in all the species.

8. The secondary phloem is moderately advanced. The sclerification of ray cells and the production of large stone cells indicates specialization. The sieve-tube elements are of the second type—with moderately oblique end walls and with nonfunctioning lattice-work on the side walls—and denote a moderate specialization relatively equal to the evolutionary level attained by the xylem. This moderate advancement supports the conclusions based on wood anatomy and floral morphology that *Casuarina* is not primitive but has been derived by specialization and reduction from a more primitive group.

9. The numerous gametophytic aberrations of *Casuarina* are discussed, and the conclusion is reached that these atypical characters are sporadic peculiarities which have little if any phylogenetic significance. Other evidence offered by nodal anatomy, cytology, paleobotany, pollen morphology, and ecology is evaluated and found to support the thesis that the Casuarinaceae are not primitive but moderately specialized.

It is a pleasure to acknowledge the guidance of Professor OSWALD TIPPO, University of Illinois, under whose supervision this research was performed. The writer is grateful to Professor GEORGE NEVILLE JONES for his suggestions in regard to the taxonomy involved in the preparation of the manuscript. The author also wishes to express his sincere appreciation for the material obtained from persons and institutions in this country and abroad. He is especially grateful to Dr. B. G. L. SWAMY of Bangalore, South India, for the loan of his manuscript on the embryogeny of *Casuarina*.

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LITERATURE CITED

1. ARBER, E. A. N., and PARKIN, J. On the origin of angiosperms. Jour. Linn. Soc. Bot. **38**:29–80. 1907.
2. ———. The relationship of the angiosperms to the Gnetales. Ann. Bot. **22**:489–515. 1908.
3. BAILEY, I. W. Microtechnique for woody structures. BOT. GAZ. **49**:57–58. 1910.
4. ———. The evolutionary history of the foliar ray in the wood of the dicotyledons and its phylogenetic significance. Ann. Bot. **26**:647–661. 1912.
5. ———. Depressed segments of oak stems. BOT. GAZ. **67**:438–441. 1919.
6. ———. The cambium and its derivative tissues. II. Size variations of cambial initials in gymnosperms and angiosperms. Amer. Jour. Bot. **7**:355–367. 1920.
7. ———. The cambium and its derivative tissues. IV. The increase in girth of the cambium. Amer. Jour. Bot. **10**:499–509. 1923.
8. ———. The problem of identifying the wood of Cretaceous and later dicotyledons: *Paraphyllanthoxylon arizonense*. Ann. Bot. **38**:439–451. 1924.
9. ———. The problem of differentiating and classifying tracheids, fiber-tracheids, and libriform wood fibers. Trop. Woods **45**:18–23. 1936.
10. ———. The development of vessels in angiosperms and its significance in morphological research. Amer. Jour. Bot. **31**:421–428. 1944.
11. BAILEY, I. W., and HOWARD, R. A. The comparative morphology of the Icacinaceae. III. Imperforate tracheary elements and xylem parenchyma. Jour. Arnold Arboretum **22**:432–442. 1941.
12. BAILEY, I. W., and SINNOTT, E. W. Investigations on the phylogeny of the angiosperms. II. Anatomical evidence of reduction in certain of the Amentiferae. BOT. GAZ. **58**:36–60. 1914.
13. BAILEY, I.W., and TUPPER, W. W. Size variation in tracheary cells. I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms, and angiosperms. Proc. Amer. Acad. Arts Sci. **54**:149–204. 1918.
14. BARGAGLI-PETRUCCI, G. Sulla structura dei legnami raccolti in Borneo. Malpighia **17**: 280–371. 1903.
15. BARGHOORN, E. S., JR. The ontogenetic development and phylogenetic specialization of rays in the xylem of dicotyledons. I. Primitive ray structure. Amer. Jour. Bot. **27**:918–928. 1940.
16. ———. The ontogenetic development and phylogenetic specialization of rays in the xylem of dicotyledons. II. Modification of the multiseriate and uniseriate rays. Amer. Jour. Bot. **28**:273–282. 1941.
17. ———. The ontogenetic development and phylogenetic specialization of rays in the xylem of dicotyledons. III. The elimination of rays. Bull. Torr. Bot. Club **68**:317–325. 1941.
18. BENSON, M. Contributions to the embryology of the Amentiferae. Part I. Trans. Linn. Soc. London, Ser. 2, **3**:409–424. 1894.
19. BENSON, M.; SANDAY, E.; and BERRIDGE, E. Contributions to the embryology of the Amentiferae. Part II. Trans. Linn. Soc. London, Ser. 2, **7**:37–44. 1906.
20. BENTHAM, G. Flora australiensis: A Description of the Plants of the Australian Territory. **6**:192–202. London. 1873.
21. BENTHAM, G., and HOOKER, J. D. Genera plantarum. L. Reeve & Co., London. 1862–1883.
22. BESSEY, C. E. The phylogeny and taxonomy of the angiosperms. BOT. GAZ. **24**:145–178. 1897.
23. ———. The phylogenetic taxonomy of flowering plants. Ann. Mo. Bot. Gard. **2**:109–164. 1915.
24. BILLINGS, F. H. Chalazogamy in *Carya olivaeformis*. BOT. GAZ. **35**:134–135. 1903.
25. BOODLE, L. A., and WORSDELL, W. C. On the comparative anatomy of the Casuarineae, with special reference to the Gnetaceae and Cupuliferae. Ann. Bot. **8**:231–264. 1894.
26. BROWN, H. P., and PANSHIN, A. J. Commercial Timbers of the United States. McGraw-Hill Book Co., Inc., New York. 1940.
27. CHALK, L. Tracheid length, with special reference to Sitka spruce (*Picea sitchensis* Carr.). Jour. Soc. Foresters Gr. Britain **4**:7–14. 1930.

28. CHALK, L. The phylogenetic value of certain anatomical features of dicotyledonous woods. Ann. Bot. (N.S.) **1**:409–428. 1937.
29. ———. Standardization of terms for vessel diameter and ray width. Trop. Woods **55**:16–23. 1938.
30. CHALK, L., and CHATTAWAY, M. M. Measuring the length of vessel members. Trop. Woods **40**:19–26. 1934.
31. ———, and ———. Factors affecting dimensional variations of vessel members. Trop. Woods **41**:17–37. 1935.
32. CHAMBERLAIN, C. J. Methods in Plant Histology. 5th ed. Univ. Chicago Press, Chicago. 1932.
33. ———. Gymnosperms, Structure and Evolution. Univ. Chicago Press, Chicago. 1935.
34. CHATTAWAY, M. M. Proposed standards for numerical values used in describing woods. Trop. Woods **29**:20–28. 1932.
35. ———. Relation between fibre and cambial initial length in dicotyledonous woods. Trop. Woods **46**:16–20. 1936.
36. CHEADLE, V. I., and WHITFORD, N. B. Observations on the phloem in the Monocotyledoneae. I. The occurrence and phylogenetic specialization in structure of the sieve-tubes in the metaphloem. Amer. Jour. Bot. **28**:623–627. 1941.
37. COMMITTEE ON NOMENCLATURE. INTERNATIONAL ASSOCIATION OF WOOD ANATOMISTS. Glossary of terms used in describing woods. Trop. Woods **36**:1–12. 1933.
38. COMMITTEE ON THE STANDARDIZATION OF TERMS OF CELL SIZE. INTERNATIONAL ASSOCIATION OF WOOD ANATOMISTS. Standard terms of length of vessel members and wood fibers. Trop. Woods **51**:21. 1937.
39. CORDEMOY, J. Contribution à l'étude de la morphologie, de l'anatomie comparée, de la phytogénie et de la biogéographie des Casuarinacées. Rev. Gen. Bot. **35**:71–91, 127–140, 186–195, 227–243, 292–303, 335–347, 399–413. 1923.
40. COULTER, J. M., and CHAMBERLAIN, C. J. Morphology of Angiosperms. Appleton & Co., New York. 1915.
41. DARRAH, W. C. Textbook of Paleobotany. D. Appleton–Century Co., New York. 1939.
42. DESCH, H. E. Significance of numerical values for cell dimensions. Trop. Woods **29**:14–20. 1932.
43. ———. Anatomical variations in the wood of some dicotyledonous trees. New Phytol. **31**: 73–118. 1932.
44. DIELS, L., and PRITZEL, E. Fragmenta phytographiae Australiae occidentalis. Bot. Jahrb. Systematik, Pflanzengeschichte und Pflanzengeographie **35**:55–662. 1904.
45. DOMIN, K. Beiträge zur flora und pflanzengeographie Australiens. Bibliotheca Bot. **89**:1–1317. 1921–1929.
46. EAMES, A. J. On the origin of the herbaceous type in angiosperms. Ann. Bot. **25**:215–224. 1911.
47. EICHLER, A. W. Syllabus der Vorlesungen über specielle und medicinisch-pharmaceutische Botanik. Berlin. 1886.
48. ENDLICHER, S. Genera plantarum secundum ordines naturales disposita. Vindobonae. 1836–1840.
49. ENGLER, A., and DIELS, L. Syllabus der Pflanzenfamilien. 11th ed. Borntraeger, Berlin. 1936.
50. ENGLER, A., and PRANTL, K. Die natürlichen Pflanzenfamilien. Engelmann, Leipzig. 1887–1915.
51. ———, and ———. Die natürlichen Pflanzenfamilien. 2d ed. Engelmann, Leipzig. 1925——.
52. ERDTMAN, G. An Introduction to Pollen Analysis. Chronica Botanica Co., Waltham, Mass. 1943.
53. ESAU, K. Development and structure of the phloem tissue. Bot. Rev. **5**:373–432. 1939.
54. ETTINGSHAUSEN, C. VON, and POKORNY, A. Physiotypia plantarum Austriacarum. Wien. 1855–1873.
55. FINN, W. W. Zur Entwicklungsgeschichte der Chalazogamen. *Ostrya carpinifolia* Scop. Jour. Inst. Bot. Acad. Sci. Ukraine **8**:15–25. 1936.
56. FROST, F. H. Specialization in secondary xylem of dicotyledons. I. Origin of vessel. BOT. GAZ. **89**:67–94. 1930.
57. ———. Specialization in secondary xylem of dicotyledons. II. Evolution of the end wall of vessel segment. BOT. GAZ. **90**:198–212. 1930.
58. ———. Specialization in secondary xylem of dicotyledons. III. Specialization of lateral wall of vessel segment. BOT. GAZ. **91**:88–96. 1931.
59. FRYE, T. C. The embryo-sac of *Casuarina stricta*. BOT. GAZ. **36**:101–113. 1903.
60. GAISER, L. O. A list of chromosome numbers in angiosperms. Genetica **8**:401–484. 1926.
61. ———. Chromosome numbers in angiosperms. II. Bibliogr. Genetica **6**:171–466. 1930.
62. ———. Chromosome numbers in angiosperms. III. Genetica **12**:161–260. 1930.
63. GARDNER, C. A. Contributiones florae Australiae occidentalis. VI. Jour. Roy. Soc. West Australia **13**:61–68. 1928.
64. GILBERT, S. J. Evolutionary significance of ring porosity in woody angiosperms. BOT. GAZ. **102**:105–120. 1940.
65. GÖPPERT, H. R. Bemerkungen über den anatomischen Bau der Casuarinen. Linnaea **15**:747–756. 1841.
66. HALLIER, H. L'Origine et le système phylétique des angiosperms exposés à l'aide de leur arbre généalogique. Arch. Néarld. Sci. Exact. et Natur., Ser. 3, **1**:146–234. 1912.
67. HEMENWAY, A. F. Studies on the phloem of

dicotyledons. II. The evolution of the sieve-tube. Bot. Gaz. **55**:236–243. 1913.

68. Houlbert, M. C. Recherches sur la structure comparée du bois secondaire dans les Apétales. Ann. Sci. Nat. Bot., Ser. 7, **17**:1–183. 1893.
69. Hutchinson, J. The Families of Flowering Plants. Macmillan & Co., London. 1926.
70. ———. A Botanist in Southern Africa. Chap. 32. P. R. Cawthorn, Ltd., London. 1946.
71. Index Kewensis plantarum phanerogamarum. Vols. 1 and 2. Supp. I–IX. 1885–1935.
72. Jeffrey, E. C. The morphology of the central cylinder in angiosperms. Trans. Can. Inst. **7**: 599–636. 1899.
73. ———. The progress of plant anatomy during the last decade. Amer. Nat. **46**:230–237. 1909.
74. ———. The Anatomy of Woody Plants. Univ. Chicago Press, Chicago. 1917.
75. ———. Technical contributions. Bot. Gaz. **86**:456–467. 1928.
76. Jeffrey, E. C., and Torrey, R. E. Transitional herbaceous dicotyledons. Ann. Bot. **35**: 227–249. 1921.
77. ———. Physiological and morphological correlations in herbaceous angiosperms. Bot. Gaz. **71**:1–31. 1921.
78. Johansen, D. A. Plant Microtechnique. McGraw-Hill Book Co., Inc., New York. 1940.
79. Johnson, A. M. Taxonomy of the Flowering Plants. Century Co., New York. 1931.
80. Jongmans, W. Fossilium catalogus. II. Plantae: Dicotyledones (ligna). Junk, Berlin. 1931.
81. Juel, H. O. Ein Beitrag zur Entwicklungsgeschichte der Samenanlagen von *Casuarina*. Flora **92**:284–293. 1903.
82. Karsten, G. Über die Entwicklung der weiblichen Blüten bei einigen Juglandaceen. Flora **90**:316–333. 1902.
83. Kribs, D. A. Salient lines of structural specialization in the wood rays of dicotyledons. Bot. Gaz. **96**:547–557. 1935.
84. ———. Salient lines of structural specialization in the wood parenchyma of dicotyledons. Bull. Torr. Bot. Club **64**:177–187. 1937.
85. Laurent, L. Les Progrès de la paléobotanique Angiospermique dans la dernière décade. Progress Rei. Bot. **1**:319–367. 1907.
86. Lecomte, H. Sur quelques pointes de l'anatomie de la tige et de la feuille des Casuarinées. Soc. Bot. France Bull. **33**:311–317. 1886.
87. Longo, B. La Mesogamia, nella commune Zucca (*Cucurbita pepo*). Rend. R. Acad. Lincei. **10**:168–172. 1901.
88. MacDaniels, L. H. The histology of the phloem in certain woody angiosperms. Amer. Jour. Bot. **5**:347–378. 1918.
89. Macklin, E. A revision of the "Distyla complex" of *Casuarina*. Trans. Proc. Roy. Soc. South Australia **51**:257–286. 1927.
90. McLaughlin, R. P. Systematic anatomy of the woods of the Magnoliales. Trop. Woods **34**:3–39. 1933.
91. Metcalfe, C. R. The systematic anatomy of the vegetative organs of the angiosperms. Biol. Rev. Camb. Phil. Soc. **21**:159–172. 1946.
92. Mez, C. Anleitung zu sero-diagnostischen Untersuchungen für Botaniker. Bot. Arch. **1**:177–200. 1922.
93. Miquel, F. A. G. *In* De Candolle, A. P., and De Candolle, A. Prodromus systematis naturalis regni vegetabilis. **16**, Pt. 2: 332–334. 1868.
94. Moll, J. W., and Janssonius, H. H. Mikrographie des Holzes der auf Java vorkommenden Baumarten. **6**:346–359. Brill, Leiden. 1936.
95. Morini, F. Contributo all' anatomia del caule e della foglia delle Casuarinee. Memoirie I. Mem. R. Accad. Sci. Inst. Bologna, Ser. 5, **4**:685–736. 1894.
96. ———. Contributo all' anatomia del caule e della foglia delle *Casuarina*. Memoirie II. Mem. R. Accad. Sci. Inst. Bologna, Ser. 5, **5**:555–615. 1895.
97. Murbeck, S. Ueber das Verhalten des Pollenschlauches bei *Alchemilla arvensis* und das Wesen der Chalazogamie. Lunds Univ. Arsskrift **36**:2 (9): 1–20. 1901.
98. Nast, C. G. The embryogeny and seedling morphology of *Juglans regia* L. Lilloa **6**:163–205. 1941.
99. Nawaschin, S. Kürzer Bericht (meiner) fortgesetzten Studien über die Embryologie der Betulineen. Ber. Deut. bot. Gesell. **12**:163–169. 1894.
100. ———. Über die gemeine Birke (*Betula alba*) und die morphologische Deutung der Chalazogamie. Mem. Acad. Imp. Sci. St. Petersbourg, Ser. 7, **42** (12): 1–40. 1894.
101. ———. Neue Ergebnisse uber Embryologie der Hasel (*Corylus avellana*). Bot. Centralbl. **63**:104–106. 1895.
102. ———. Ein neue Beispiel der Chalazogamie. Bot. Centralbl. **63**:353–357. 1895.
103. ———. Über das Verhalten des Pollenschlauches bei der Ulme. Bull. Acad. Imp. Sci. St. Petersbourg, Ser. 5, **8** (5): 345–357. 1898.
104. ———. Ueber die Entwicklung der Samenknopse und den Weg des Pollenschlauches bei *Alnus viridis*. Bot. Centralbl. **77**:106. 1899.
105. ———. Zur Entwicklungsgeschichte der Chalazogamen, *Corylus avellana* L. Bull. Acad. Sci. St. Petersbourg, Ser. 5, **10**:375–391. 1899.
106. Nawaschin, S., and Finn, W. W. Zur Entwicklungsgeschichte der Chalazogamen, *Juglans regia* und *Juglans nigra*. Mem. Acad. Imp. Sci. St. Petersbourg, Ser. 8, **31** (9): 1–59. 1913.
107. Parkin, J. The evolution of the inflorescence. Jour. Linn. Soc. Bot. **42**:511–563. 1914.
108. Poisson, J. Recherches sur les *Casuarina* et en particulier sur ceux de la Nouvelle-Calédonie. Paris. 1876.
109. Praël, E. Vergleichende Untersuchungen über

Schutz- und Kern-Holz der Laubbaüme. Jahrb. wiss. Bot. **19**:1–81. 1888.

110. RECORD, S. J. Role of wood anatomy in taxonomy. Trop. Woods **37**:1–9. 1934.
111. ———. Identification of the Timbers of Temperate North America. Wiley & Sons, Inc., New York. 1934.
112. ———. Classification of various anatomical features of dicotyledonous woods. Trop. Woods **47**:12–27. 1936.
113. RECORD, S. J., and CHATTAWAY, M. M. List of anatomical features used in classifying dicotyledonous woods. Trop. Woods **57**:11–16. 1939.
114. RENDLE, A. B. A new group of flowering plants. Nat. Sci. **1**:132–143. 1892.
115. ———. The Classification of the Flowering Plants. Vols. **1** and **2**. Cambridge, England. 1925.
116. RENDLE, B. J. Gelatinous wood fibers. Trop. Woods **52**:11–19. 1937.
117. RENDLE, B. J., and CLARKE, S. H. The problem of variation in the structure of wood. Trop. Woods **38**:1–8. 1934.
118. ———, and ———. The diagnostic value of measurements in wood anatomy. Trop. Woods **40**:27–37. 1934.
119. ROBERTSON, C. The structure of the flowers and the mode of pollination of the primitive angiosperms. BOT. GAZ. **37**:294–298. 1904.
120. SCHNARF, K. Embryologie der Angiospermen. Handbuch Pflanzenanatomie herausg. von K. LINSBAUER. Abt. II, Teil 2. Berlin. 1929.
121. ———. Contemporary understanding of embryo-sac development. Bot. Rev. **2**:565–585. 1936.
122. SHOEMAKER, D. N. On the development of *Hamamelis virginiana*. BOT. GAZ. **39**:248–266. 1905.
123. SINNOTT, E. W. Investigations on the phylogeny of the angiosperms. I. The anatomy of the node as an aid in the classification of angiosperms. Amer. Jour. Bot. **1**:303–322. 1914.
124. SINNOTT, E. W., and BAILEY, I. W. The significance of the "foliar ray" in the evolution of herbaceous angiosperms. Ann. Bot. **36**:523–583. 1922.
125. SOLEREDER, H. Systematic Anatomy of the Dicotyledons. Trans. L. A. BOODLE and F. E. FRITSCH. Oxford. 1908.
126. STEENIS, C. G. G. VAN. On the occurrence of *Casuarina nodiflora* Forst. in Australia. Jour. Arnold Arb. **12**:197. 1931.
127. SWAMY, B. G. L. A contribution to the life-history of *Casuarina*. (Unpublished manuscript.)
128. THOMPSON, W. P. On the origin of the multiseriate ray of the dicotyledons. Ann. Bot. **25**:1005–1014. 1911.
129. TIPPO, O. Comparative anatomy of the Moraceae and their presumed allies. BOT. GAZ. **100**:1–99. 1938.
130. ———. A list of diagnostic characteristics for descriptions of dicotyledonous woods. Trans. Ill. Acad. Sci. **34**:105–106. 1941.
131. ———. The role of wood anatomy in phylogeny. Amer. Midl. Nat. **36**:362–372. 1946.
132. TISCHLER, G. Pflanzliche Chromosomen-Zahlen. Tab. Biol. Period. **4**:1–83. 1927.
133. ———. Pflanzliche Chromosomen-Zahlen. Tab. Biol. Period. **7**:109–226. 1931.
134. TREUB, M. Sur les Casuarinées et leur place dans le système naturel. Ann. Jard. Bot. Buitenzorg **10**:145–231. 1891.
135. TURRILL, W. B. Taxonomy and phylogeny. Part II. Bot. Rev. **8**:473–532. 1942.
136. VUILLEMIN, P. Les Chalazogames de M. Treub et l'évolution des Phanérogames. Rev. Gén. Sci. Pur. Appl. **3**:22–24. 1892.
137. WANSCHER, J. H. The basic chromosome numbers of the higher plants. New Phytol. **33**:101–126. 1934.
138. WARD, L. F. The Potomac Formation. 15th. Ann. Rep. U.S. Geol. Surv.: 313–397. 1895.
139. WETMORE, R. H. The use of celloidin in botanical technic. Stain Technol. **7**:37–62. 1932.
140. WETTSTEIN, R. Handbuch der systematischen Botanik. 4th ed. Deuticke, Leipzig and Wien. 1935.
141. WODEHOUSE, R. P. Pollen Grains. McGraw-Hill Book Co., Inc., New York. 1935.
142. WOODROOF, J. G., and WOODROOF, N. C. The development of the pecan-nut (*Hicoria pecan*) from the flower to maturity. Jour. Agr. Res. **34**:1049–1063. 1927.
143. ZINGER, N. Beiträge zur Kenntnis der weiblichen Blüten und Infloresenzen der Cannabineen. Flora **85**:189–292. 1896.
144. ZITTEL, K. A. Handbuch der Paleontologie. Abt. II. Palaeophytologie. Oldenbourg, München, and Leipzig. 1890.

A STUDY OF THE PLASTIDS IN THE CELLS OF THE MATURE SPOROPHYTE OF ISOETES

WILSON N. STEWART

Introduction

STRUCTURE OF PLASTIDS.—To early workers chloroplasts appeared optically homogeneous. MEYER (**17**) demonstrated with the aid of living preparations that the chloroplasts of *Acanthophippium* possess an almost colorless ground substance (stroma) in which are imbedded chlorophyll-containing granules (grana—fig. 1). This view was supported by the researches of SCHIMPER (**20**) but was not generally accepted until more recently when HEITZ (**8**) and WEIER (**23, 24, 25**) observed the grana in the chloroplasts of many different species of plants.

According to HEITZ (**8**), the grana are not spherical but are plate-like structures ranging from 0.5 to 2.0 μ in diameter and are aligned in regular rows. This alignment frequently gives the appearance of fibrils, a fact which has been offered as an explanation of the fibrillar structure of the chloroplast described by HABERLANDT (**7**).

YUASA (**26**) studied *in vivo* the chloroplasts of *Pteris multifida*, *Adiantum capillus—veneris*, *Athyrium nipponicum*, *Selaginella uncinata*, and *S. kraussiana* and described them as having a thin external membrane and an inner ground substance containing a chlorophyll network. The chlorophyll network in turn is differentiated into a linear arrangement of grana and connecting threads (fig. 2). He showed the network lying well within the stroma of the plastid apart from the plastid membrane. The grana described by YUASA are spindle-shaped structures 0.5–2.0 μ in length.

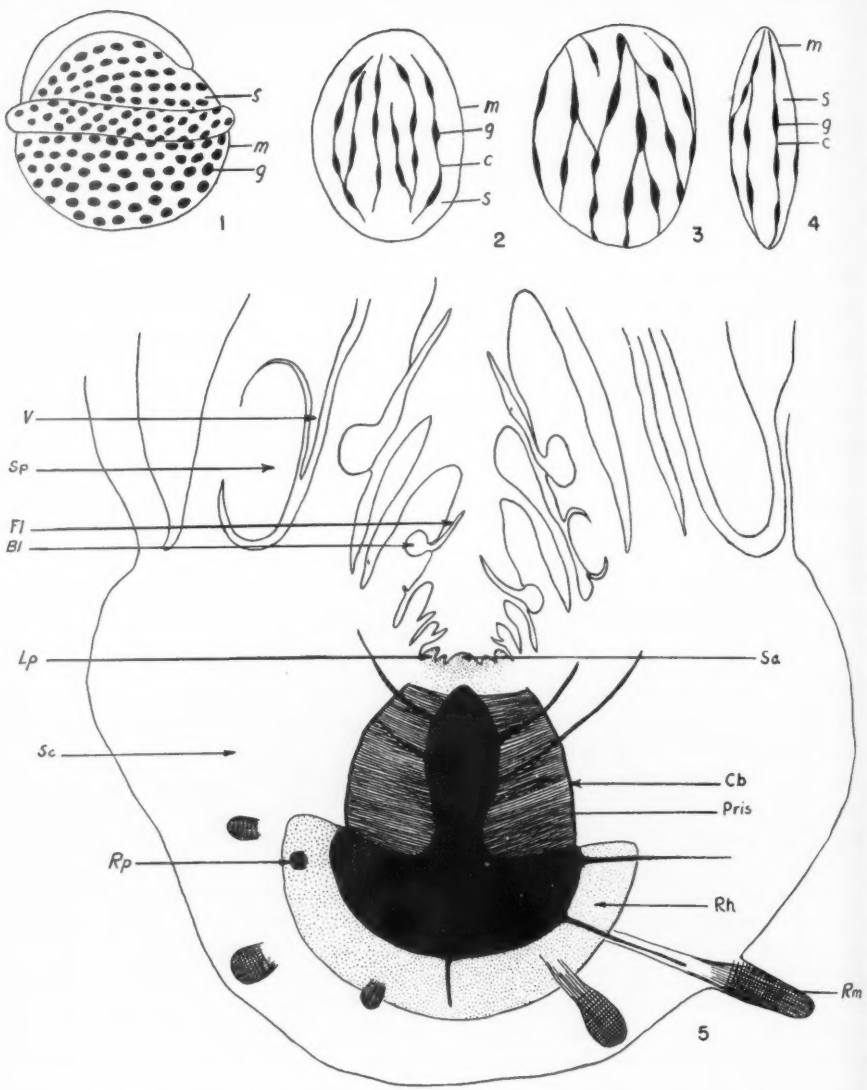
ZIRKLE (**28**) investigated the structure of the chloroplasts of several spermatophytes, pteridophytes, bryophytes, and green algae, and observed "colorless granules" in chloroplasts seen in face view. He interpreted these granules as the ends of pores which pass through the stroma and connect with a larger central vacuole of the plastid.

The citations given above include the major concepts of plastid structure. It was one of the purposes of the present investigation to determine the structure and function of the plastids of *Isoetes*.

PLASTID POLARITY.—If a single plastid of a meristematic cell divides to form two (or four) daughter plastids, each of which functions in the formation of the achromatic figure as they migrate to the poles of the cell and each of which persists in one of the daughter cells after nuclear and cell division, a cell is said to exhibit "plastid polarity" (**4**). This type of plastid behavior has been described as occurring in the meristematic cells of many pteridophytes and bryophytes (**1, 3, 4, 5, 6, 9, 16, 18, 19, 21**).

The behavior of the plastids in vegetative cells of *Isoetes* was first described by MARQUETTE (**13**), who observed a single "polar structure" in each cell of the intercalary meristem of the leaf of *I. macrospora*. The "polar structure" was shown to exhibit plastid polarity. DUNLOP's (**4**) more recent description of plastid behavior in the root tips of *I. muricata* var. *braunii* is similar to MARQUETTE's description for the leaf meristem.

It was evident that thus far there were



FIGS. 1–5.—Figs. 1–4, chloroplast types. Fig. 1, chloroplast of *Acanthophippium; s*, stroma; *m*, membrane; *g*, grana. (After MEYER.) Fig. 2, chloroplast of *Selaginella; c*, connecting fibers. (After YUASA.) Fig. 3, chloroplast of *Isoetes muricata* var. *braunii;* surface view. Fig. 4, chloroplast of *I. muricata* var. *braunii*, side view. Fig. 5, diagram of longisection through mature sporophyte of *Isoetes; V*, velum; *Sp*, sporangium, *Fl*, blade of ligule; *Bl*, base of ligule; *Lp*, leaf primordium; *Sc*, secondary parenchyma; *Rp*, root primordium; *Sa*, stem apex; *Cb*, anomalous cambium; *Pris*, prismatic layer; *Rh*, rhizophore meristem; *Rm*, root meristem.

descriptions of plastid polarity for only two regions in the vegetative sporophytic tissues of *Isoetes* and that many of these tissues remained to be examined. Thus the second purpose of this investigation was to make an examination of all the meristematic vegetative tissues of the sporophytes of available species of *Isoetes*, to determine whether their plastids assume polar positions.

Material and methods

Two species of *Isoetes* were used. *I. macrospora* was collected from Devil's Lake, Sauk County, Wisconsin. *I. muricata* var. *braunii* was found growing in Little Crooked Lake and Boulder Lake, Vilas County, Wisconsin.

A collection of nearly a hundred living plants of *I. muricata* var. *braunii* was brought to Madison, planted in tanks and maintained under greenhouse conditions. Half of them were planted in fine sand from the lake bottom in which they originally grew. The remainder was planted in a mixture of sand and fine gravel obtained from the greenhouse supply. All plants were covered with about 18 inches of slowly circulating lake water. Only those plants maintained in their natural substrate showed continued vigorous growth.

The healthy plants were used for studies *in vivo* of the structure of the chloroplast. Free-hand sections were made of the leaves with a razor blade. The structure of the chloroplast is very evident in material mounted in water and remains unaltered when either 7.5% sucrose or physiological saline solution is used. Graham's iodine was used to determine the position of the starch within the chloroplast.

Collections of material for fixation were made of both species of *Isoetes* in the summers of 1938, 1940, and 1941. The killing fluids used included Belling's, Flemming's medium and strong, chromacetic *a* and *b*, Randolph's, and F.A.A. Dehydration and infiltration series of ethyl alcohol and chloroform were used; the material thus treated was imbedded in "Parowax." Sections cut 8–10 μ thick were stained with one of the following combinations: picric acid and crystal violet, Flemming's triple stain, or Heidenhein's iron-alum haematoxylin.

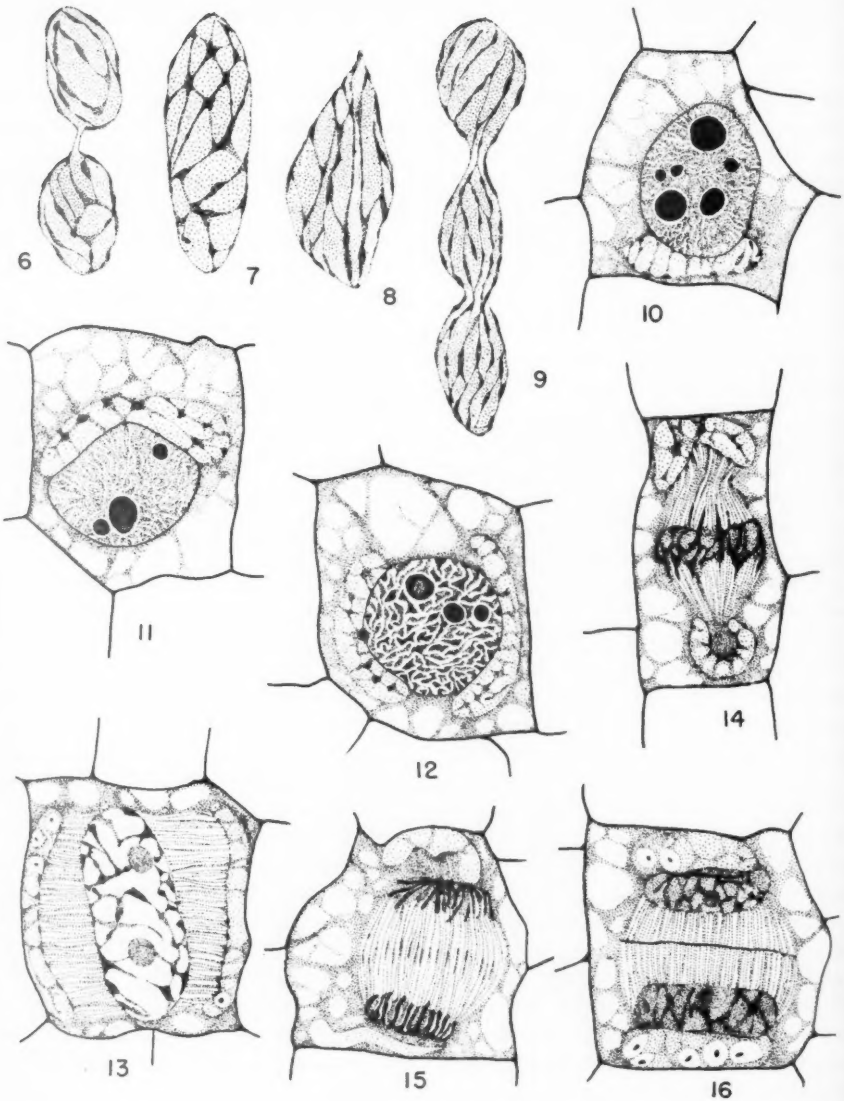
During the course of this study serial sections of all the tissues of the mature sporophyte of both species of *Isoetes* were made. Both living and fixed material were used in studying the structure of the plastid.

Observations

Structure of plastids

Chloroplasts.—Chloroplasts seen *in vivo* in the mature leaf cells of *Isoetes muricata* var. *braunii* are ovoid. Their average dimensions in face view are 7.8 μ in length, 5.3 μ in width; in side view the average width is 3 μ. Each chloroplast is bounded by what appears to be a membrane (figs. 3, 4). Distributed through the stroma is a distinct network consisting of grana and connecting threads (figs. 3, 4, 6–9). The grana of the chloroplast are linearly arranged, spindle-shaped structures averaging 0.85 μ in length and 0.4 μ in width. The distribution of the chlorophyll within the plastid was not determined with certainty. It appeared to be associated with the stroma as well as with the network of the plastid.

The most obvious difference between chloroplasts seen in a living cell and those in a fixed cell is the absence of chlorophyll from the latter. The grana and connecting fibers of the network undergo little change when a chloroplast is fixed (cf. figs. 7 and 23 with figs. 6, 8, and 9).



FIGS. 6–16.—Figs. 6, 8, 9, *I. muricata* var. *braunii* (camera lucida). Figs. 7, 10–16, *I. macrospora* (camera lucida). Figs. 6, 8, 9, chloroplasts from leaf cells as observed in free-hand sections. ×2270. Fig. 7, chloroplast as observed in killed and stained preparation. ×2270. Fig. 10, "resting" cell in intercalary meristem of leaf. ×1200. Figs. 11–16, stages of nuclear, cell, and plastid division in cells of intercalary meristem of leaf. ×1200.

The membrane of the chloroplast in killed and sectioned material was stained by either boiled Orange G, or an alcoholic solution of fast green, or Heidenhein's iron-alum haematoxylin. The intensity of the stain of the membrane is not much greater than the intensity of that taken by the surrounding dense cytoplasm and by no means equals the intense staining reaction of the nuclear membrane. The network of grana and connecting fibers takes a deep stain with either gentian violet or Heidenhein's haematoxylin.

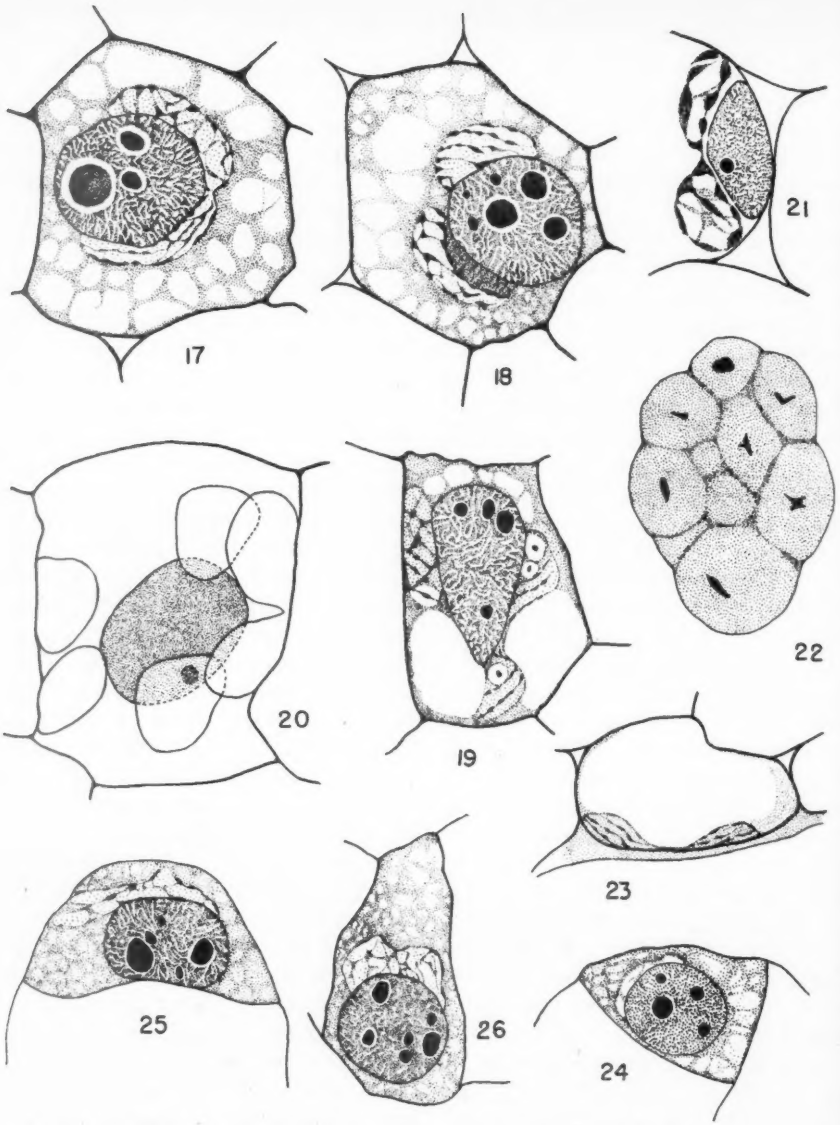
LEUCOPLASTS.—Unlike the chloroplasts, the leucoplasts are extremely variable in form. In size and shape the leucoplasts differ according to the sporophytic tissue in which they occur. Each meristematic cell, as seen in the metabolic stage, contains but one plastid which is tightly appressed to one side of the nucleus, covering approximately one-third of the nuclear surface (figs. 10, 24, 25, 26, 30, 37, 49). This plastid, like the chloroplast, has a delicate membrane which delimits it from the surrounding cytoplasm. Within the plastid can be seen fine threads extending between small granules (figs. 10, 25, 26, 30, 37, 49). Frequently the delicate threads are seen to extend across the width of the plastid from that side appressed to the nucleus to the side adjoining the cytoplasm (figs. 10, 49).

As the cells of the meristematic regions mature, the single plastid in each may enlarge as the number of starch grains increases, and it becomes a well-defined leucoplast (figs. 21, 22, 27, 45, 46). Frequently associated with each small starch grain in the leucoplast is a minute refractive body which stains red with the triple stain (figs. 19, 45, 46). A layer of violet-staining starch surrounds each such body. In a larger starch grain the position earlier occupied by the refractive body is represented by the hilum (figs. 22, 58). The increase in the size of the starch grain is accompanied by an increase in the size of the refractive hilum. Figure 58 shows the typical non-laminated starch grains of a storage cell.

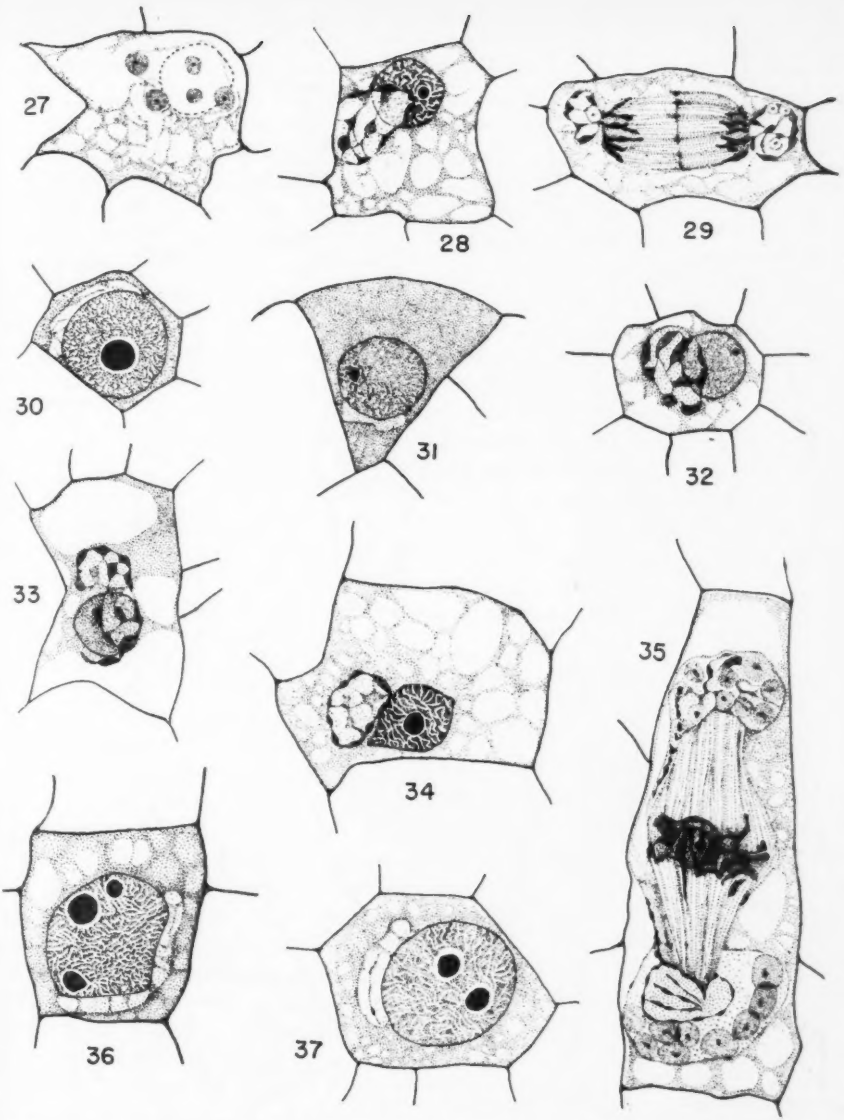
POLAR BEHAVIOR OF PLASTIDS

LEAF.—The leaf primordium owes its origin to the activity of an apical cell which occupies the base of a cuplike depression in the apical region of the stem. In the center of the depression lies the meristem of the stem apex (fig. 5), and the leaf primordia are distributed spirally about this meristematic region. On the sides of the depression and also spirally arranged are the leaves in different stages of differentiation, the youngest nearest the center, the oldest toward the periphery. An older, differentiated leaf has an intercalary meristematic region above the ligule. The cells of the upper portions of this meristem mature into the green photosynthetic leaf cells; a few cells in the lower portion mature into colorless starch-storage cells.

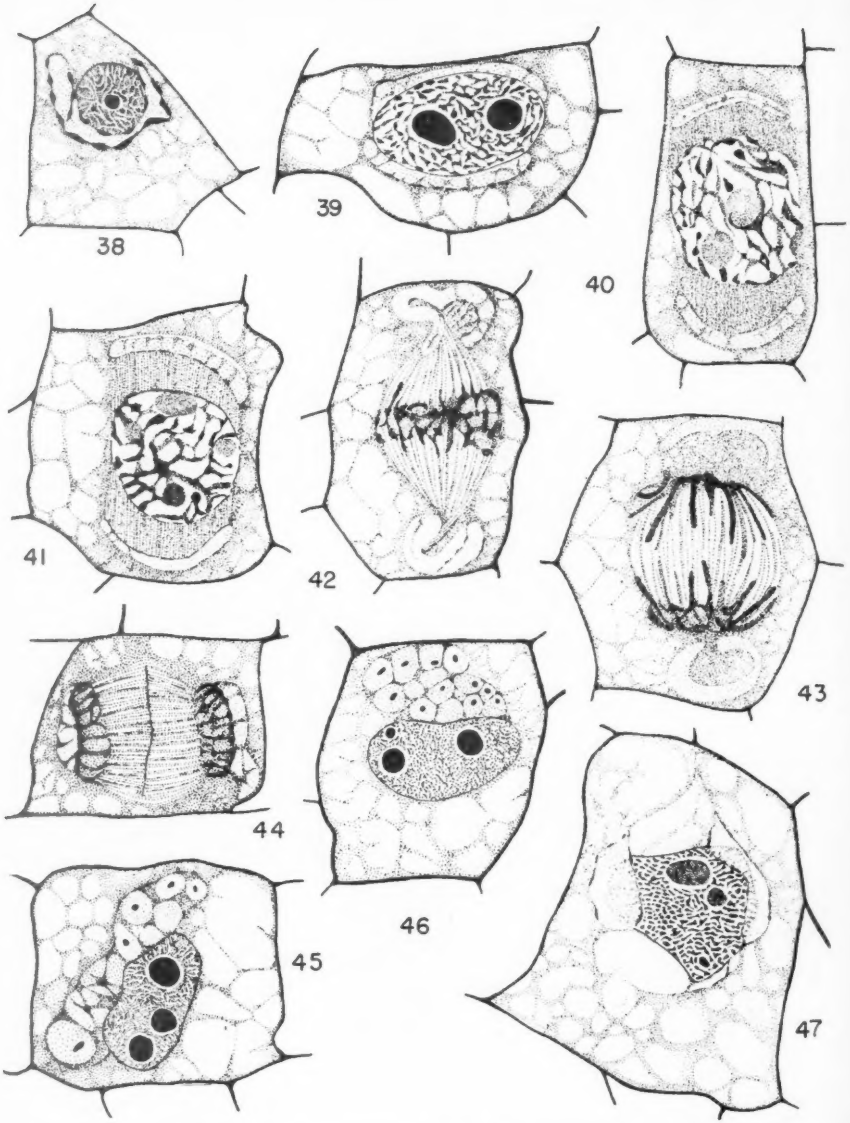
Within each "resting" cell of the intercalary meristem is a single plastid. This is an irregular, somewhat cup-shaped structure whose concave surface is appressed to the nucleus (fig. 10). The association between plastid and nucleus is so intimate that the plastid often fits into a depression on the surface of the interphase nucleus. In some cells at the metabolic phase, it may be observed that the plastid has elongated and that a constriction has appeared at its center (figs. 11, 17). The direction of elongation of the plastid is not always parallel to the longitudinal axis of the cell; it may be at right angles or diagonal to this axis. The elongating plastid in time divides to form two daughter plastids each of which moves



FIGS. 17–26.—Figs. 17–20, 22, 24–26, *I. macrospora* (camera lucida). ×1200. Figs. 21, 23, *I. muricata* var. *braunii* (camera lucida). ×1200. Figs. 17–19, cells from maturation region of leaf above intercalary meristem. Fig. 20, older cell of leaf showing position of five plastids. Fig. 21, portion of cell from maturation region below intercalary meristem of leaf. Fig. 22, single leucoplast from nonphotosynthetic cell of mature leaf base. Fig. 23, epidermal cell of mature leaf. Fig. 24, apical cell of leaf primordium. Fig. 25, terminal cell of young ligule. Fig. 26, basal cell of young ligule.



FIGS. 27–37.—Figs. 27–34, 37, *I. muricata* var. *braunii* (camera lucida). Figs. 35, 36, *I. macrospora* (camera lucida). ×1200. Figs. 27, 28, cells from base of mature ligule. ×1200. Fig. 29, cell from immature velum. ×1200. Fig. 30, cell from potential sporogenous tissue. ×1670. Fig. 31, tapetal cell from microsporangium. ×1670. Fig. 32, cell of microsporangium wall. ×1200. Fig. 33, cell from trabecula of microsporangium. ×1200. Fig. 34, "resting" cell in rhizophore meristem. ×1200. Fig. 35, stage of nuclear and plastid division in cell of rhizophore meristem. Fig. 36, "resting" cell of root meristem. Fig. 37, large "resting" cell of stem apex meristem. ×1200.



FIGS. 38–47.—Fig. 38, "resting" cell of stem apex meristem about 30 μ back from meristem surface. *I. muricata* var. *braunii* (camera lucida). ×1200. Figs. 39–47, *I. macrospora* (camera lucida). ×1200. Figs. 39–44, stages of nuclear, cell, and plastid division in cells of root meristem. Figs. 45, 46, cells of maturation region of root. Fig. 47, cell of anomalous cambium showing early stages of division of nucleus and plastid.

along the surface of the nucleus until they lie on opposite sides of the nucleus (fig. 12). By this time the nucleus has entered the prophase. While the daughter plastids are lying at opposite sides of the prophase nucleus, they elongate until each is nearly twice as long as the original mother plastid. What the daughter plastids gain in length they may lose in width.

The daughter plastids then move away from the membrane of the prophase nucleus toward opposite poles of the cell, either in a direction along the longitudinal axis of the cell or, in many instances, obliquely or at right angles to this axis. As they move away from the nucleus, the space left between the inner surface of each daughter plastid and the nuclear membrane is occupied by spindle fibers (fig. 13). At this point the nucleus is in late prophase. After the nuclear membrane disappears, spindle fibers are seen to connect the inner surface of the daughter plastids. The spindle poles are extremely broad at this stage, occupying the full extent of the inner surface of each daughter plastid (fig. 13).

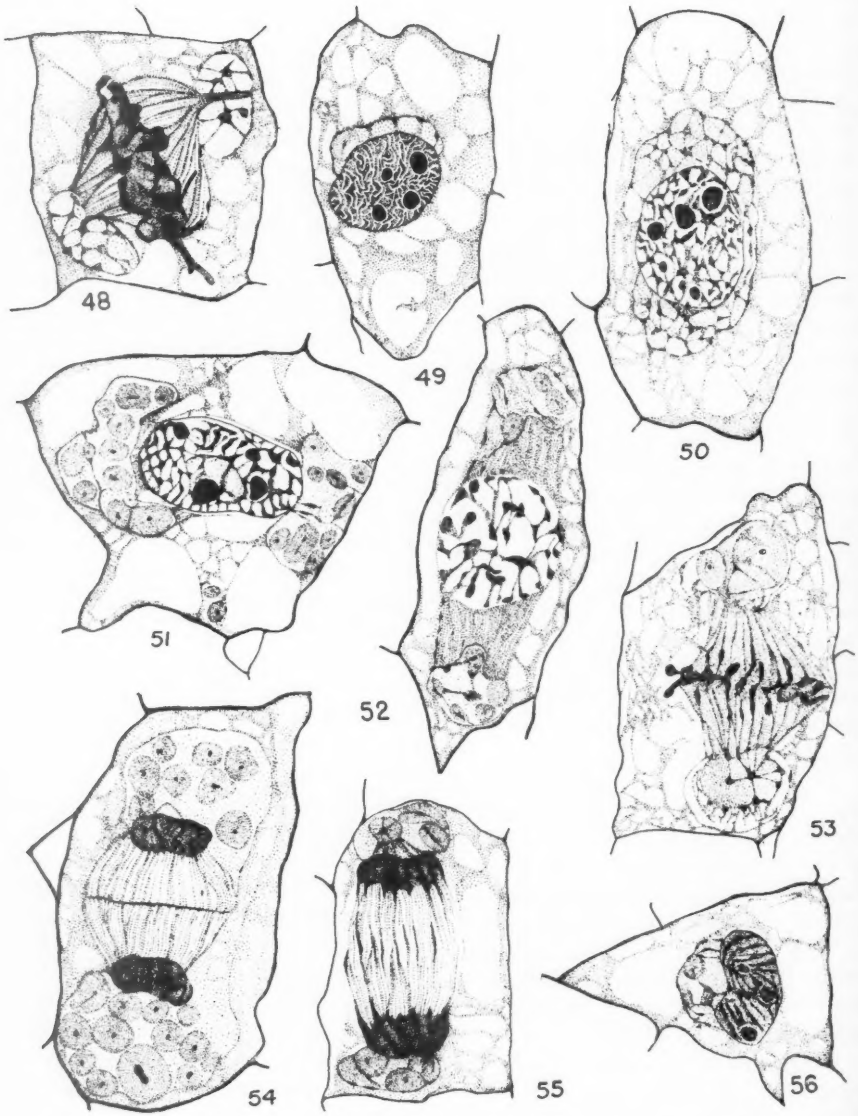
It is evident that the polar positions assumed by the daughter plastids within the cell are in accord with the orientation of the achromatic figure. If the plastids lie at the poles of the longitudinal axis of the cell, the spindle will lie on, or parallel to, this axis. If the daughter plastids occupy other positions, the achromatic figure will be oblique or at right angles to the longitudinal axis.

While the chromosomes are in the equatorial plate, the plastids at the spindle poles shorten, and their margins bend toward the equator of the cell; the plastids now appear in sectional view as thick crescent-shaped structures (fig. 14). The cumulative effect of the shortening and crenation of the plastids is that the spindle fibers which are attached to the inner surfaces are crowded together; the achromatic figure now appears pointed at each pole (fig. 14).

During late anaphase and early telophase the plastids lose something of their curvature, becoming flatter; the spindle poles are now less sharply pointed and less distinct than at earlier stages (fig. 15). The straightening of the daughter plastids causes them to assume dimensions approaching those which earlier characterized the mother plastid. Each daughter plastid becomes appressed to the new daughter nucleus formed at the pole of the cell occupied by the plastid (fig. 16).

Cell division is initiated at telophase by the formation of a cell plate at the equator of the spindle (figs. 15, 16). At the completion of cell division, each daughter cell contains a nucleus with a plastid appressed to it.

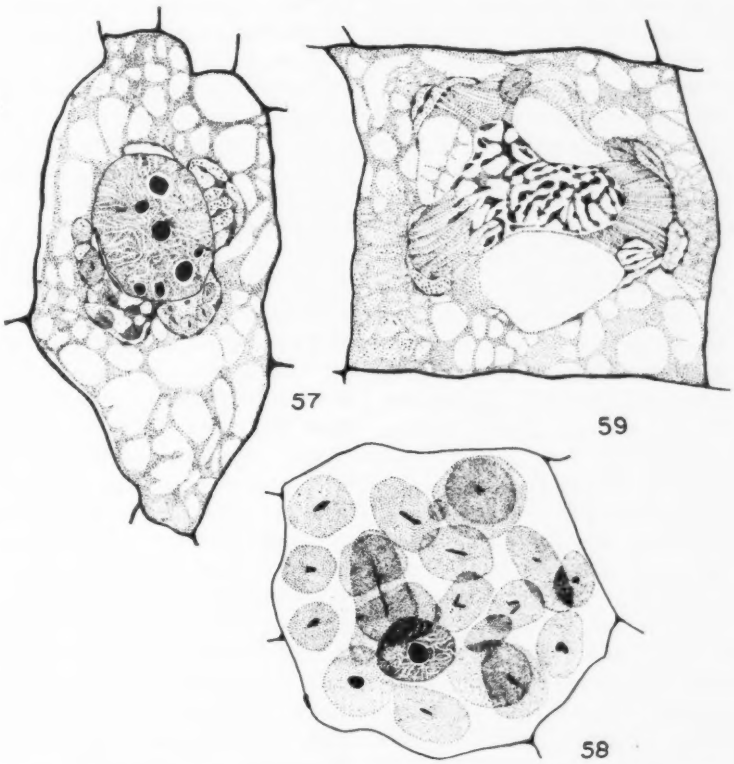
The division of a single plastid to form two daughter plastids, followed by nuclear division, spindle formation, cell division, and, finally, the organization of two daughter cells, each containing a single plastid, may be repeated for many cell generations within the intercalary leaf meristem. In the area of transition above the intercalary meristem, where cells are maturing, there are many cells which contain from one to six plastids each (figs. 19, 21). Many of these plastids show no contact with the nucleus, yet they continue to divide, forming more daughter plastids. Close observation of those cells containing one plastid reveals that the division of the plastid occurs as described for the meristematic cells but that, as the daughter plastids move away from the nuclear membrane, there is no indication of the onset of nuclear division (fig. 18). From this point on in the devel-



FIGS. 48–56.—*I. macrospora* (camera lucida). ×1200. Fig. 48, cambial cell at metaphase showing pointed spindle. Fig. 49, "resting" cell of secondary parenchyma. Figs. 50–56, stages of nuclear, cell, and plastid division in cells of secondary parenchyma.

opment of the leaf cell any association between nucleus and plastid is random.

As the leaf cells mature, they are pushed upward and become exposed to the light. Their plastids become the photosynthetic chloroplasts of the mature cells. The older the cell, the greater is the number of chloroplasts, so that in the older photosynthetic leaf cells of *Isoetes muricata* var. *braunii* there may be as many as twenty-nine chloroplasts per cell. Many stages in the division of the chloroplasts have been observed in living cells (figs. 6, 9). Although the shape of the chloroplast is less variable than that of the plastid of a meristematic cell, the method of division by constriction is common to both. First the chloroplast grows in length, then a constriction appears near its center. The constriction increases, leaving a narrow isthmus and resulting in a dumbbell shape (fig. 6). Often before the constriction is complete the dividing plastid may continue to elongate, and a new constriction appears (fig. 9). Elongation of the chloroplast prior to division is apparently accompanied by an increase in the number of



FIGS. 57–59.—*I. macrospora* (camera lucida). ×1200. Fig. 57, maturing cell of secondary parenchyma. Fig. 58, mature cell of secondary parenchyma. Fig. 59, tripolar spindle in cell of anomalous cambium.

grana and connecting threads, both of which are approximately equally divided among the daughter plastids.

Relatively few cells from the lower portion of the intercalary meristem undergo maturation. The few that mature do not become photosynthetic but remain colorless because they are covered by the substrate in which the plant grows. Their plastids behave somewhat differently from those of the cells destined to become photosynthetic. Frequently the single plastid observed in each cell of the intercalary meristem does not divide again as the cell matures, so that only one plastid is present in the mature cell. Sometimes two to four plastids occur in mature cells, indicating that the mother plastid of the meristematic cell has divided. Each of these plastids becomes filled with starch and has the characteristic appearance of a leucoplast (fig. 22).

The "resting" apical cell present in the early development of the leaf possesses a single plastid (fig. 24). This plastid is not so well defined or so large as the plastids in the "resting" cells of the intercalary meristem of the leaf, but its relation to the nucleus is similar. It divides to form two daughter plastids each of which occupies one pole of the cell, later becoming the plastid of a daughter cell. This same behavior is repeated in the cells of the older leaf primordia.

The ligule (fig. 5) differentiates at a relatively early stage in the development of the leaf and in turn becomes differentiated into a basal portion imbedded in the tissues of the young leaf and a triangular bladelike portion which at maturity is one to three cells in thickness. The basal cell of the young ligule contains a well-defined plastid appressed to the interphase nucleus (fig. 26); the terminal cell of the blade likewise possesses a single, irregularly shaped plastid (fig. 25).

There is generally only one plastid in each cell of the mature ligule. Figures 27 and 28 show cells from its basal portion with one leucoplast per cell. The cells of the blade produce large quantities of mucilaginous material which escapes from these cells, but they never possess starch-containing plastids. It is possible that the single plastid present in each meristematic ligule cell may play a part in the formation of the mucilaginous material as these cells mature.

A ligule sheath surrounds that portion of the ligule which is imbedded in the tissues of the leaf. This sheath is one cell in thickness; the radial walls of these cells have thickenings comparable with the Casparian strips found in an endodermis. The nuclei of the sheath cells are small and dark-staining as compared with the nuclei of other leaf cells. There is little or no cytoplasm visible, nor is there any indication of a plastid. It is suggested that the cells of the ligule sheath are dead at maturity.

Any leaf of the plant may be a sporophyll, bearing either a micro- or a megasporangium (fig. 5). The potential sporogenous tissue is differentiated from the other leaf cells early in the development of the leaf. The amount of such tissue increases rapidly by frequent mitotic divisions. Each "resting" cell of this tissue, like the "resting" cells of other meristematic regions of the leaf, has a single small plastid appressed to the nucleus (fig. 30). At the time of division the behavior of this plastid is similar to that of the plastids described in the other meristematic tissues.

The potential sporogenous cells differentiate into the tissues of a micro- or megasporangium. These tissues include

the sporangium wall, the trabeculae, the tapetum, and the sporocytes. Each cell of the sporangium wall and those of the trabeculae has a single large leucoplast associated with the nucleus (figs. 32, 33). Each tapetal cell may also contain a single plastid appressed to the nucleus (fig. 31), but it is small and difficult to distinguish.

A delicate membrane—the velum—covers half to two-thirds of the exposed surface of the sporangium (fig. 5). Here again a leucoplast is associated with the nucleus of each cell. A division figure found in a comparatively young velum shows a leucoplast at each pole (fig. 29), indicating that the plastid goes through the same sequence as has been described for the plastids in other meristematic regions.

MERISTEM OF STEM APEX.—The apical growing-point of the stem lies in the center of the cup-shaped depression on the upper surface of the "corm" (fig. 5). It is characterized by a mound of relatively large meristematic cells, the nuclei of which are larger than those of other cells. Each cell of the stem apex meristem has a plastid lying next to the nucleus (fig. 37); the plastid is similar in appearance to the plastids described in other meristematic "resting" cells. Within the cells of the apical meristem which lie about 30 μ back of the meristematic hump there are plastids, one per cell, which contain many small starch grains (fig. 38).

RHIZOPHORE MERISTEM.—This lies parallel to and above the furrow formed by the line of union of the two lateral lobes of the "corm" (fig. 5). Like the cells of the meristem of the stem apex, the cells of the rhizophore meristem seldom divide. Associated with each interphase nucleus is a single plastid containing several minute starch grains (fig. 34). Dividing cells of the rhizophore meristem frequently have large numbers of relatively large starch grains in the single plastid. The presence of the starch grains within the plastid may hinder its division to a certain extent. In one division figure the chromosomes were at metaphase, and the daughter plastids occupied the respective poles. Each daughter plastid contained many starch grains and had long extensions toward the equator (fig. 35). The latter were taken as an indication that the division of the mother plastid had just been completed. This is in contrast to the condition in other meristematic tissues, in which plastid division is completed while the nucleus is still in early prophase.

ROOT.—The root primordium originates from an apical cell located in the rhizophore meristem (fig. 5). As in the leaf, the apical cell soon gives way to a group of meristematic cells which in turn undergo rapid division to form the meristematic region of the root. The primordium pushes downward and to the outside of the "corm" through the intervening parenchyma (fig. 5).

The sequence of events concerned in the division of the plastid in a meristematic cell of the root tip (figs. 39–44) is similar to that previously described in the meristematic cells of the leaf, in the meristem of the stem apex, and in the rhizophore meristem.

The elongation and constriction of the plastid is often accompanied by a change in shape of the "resting" nucleus. The side of the nucleus next to the elongating plastid is no longer rounded but has two flattened surfaces forming a rooflike angle (fig. 36). The region of constriction of the plastid lies opposite the peak of the nucleus. This type of nuclear and plastid configuration is frequently observed in

other meristematic tissues of the sporophyte (fig. 11).

Figure 40 illustrates the accumulation of granular material lying between the nuclear membrane and the inner face of the daughter plastids which is destined to become the achromatic figure.

Cells which are beginning to mature just back of the meristematic region of the root have one or two large starch-containing plastids. Each starch grain has a single refractive, red-staining hilum (figs. 45, 46). Many stages in the division of the plastids may be seen in the cells of the maturation region of the root. The division of the plastid in a cell in this region does not initiate a division of the nucleus, nor do the daughter plastids formed occupy the polar regions of the cell (figs. 45, 46). Instead, the daughter plastids are variously located in the cell. In the mature root the parenchymatous cells of the outer and inner cortex contain one to four starch-filled plastids which resemble the leucoplasts of mature non-photosynthetic leaf cells (fig. 22).

ANOMALOUS CAMBIUM.—The anomalous cambium is peculiar to the stem of *Isoetes;* none of the other living Lycopsida has this characteristic tissue. It is a ring of flattened meristematic cells surrounding the protostele (fig. 5) and extends from a point just below the youngest leaf traces of the stem to the edge of the meristematic region of the rhizophore. The cambium forms the cells of the prismatic layer to the inside and the cells of the secondary cortex to the outside (fig. 5).

The cells of the prismatic layer are arranged in radial rows. The nature of these cells has not been determined with certainty. They are apparently devoid of cytoplasm at maturity, and therefore of plastids, but contain a small dark-staining nuclear remnant.

The lateral extension of the two lobes of the "corm" is dependent upon the formation of the cells of the secondary parenchyma by the cambium and the subsequent divisions of the first-formed cells of the secondary parenchyma.

Each of the "resting" cambial initials has a small, poorly differentiated plastid, which is difficult to distinguish because the nucleus is large in proportion to the size of the cell. In consequence the plastid is frequently pressed between the nucleus and the cell wall. The presence of the plastid becomes obvious when it divides. Division follows the same sequence as described for the division of plastids in the other meristematic tissues of the sporophyte. Figure 47 shows the two daughter plastids of a cambial initial at opposite sides of a prophase nucleus. The margins of one of the daughter plastids seen in figure 48 are folded toward the equatorial region to such an extent as to envelop a portion of the pointed spindle.

Cells formed to the outside by divisions of the cambial initials retain their capacity for nuclear and cell division. The single plastid within each meristematic "resting" cell of this outer region is, as already described for other meristematic regions, appressed to one side of the nucleus (fig. 49). The plastids of these cells differ from those of the cambial initials in that they are better defined and frequently contain a number of large starch grains.

The sequence of plastid division as related to the sequence of nuclear division in the meristematic cells to the outside of the cambium differs from that described for other tissues. The plastid elongates until its two lobes lie at opposite sides of the nucleus (fig. 50). The division of the mother plastid into two daughter plastids is delayed, and a connecting isthmus between the two daugh-

ter plastids may be present during all the prophase stages (figs. 50–52). This delay in separation may be due to the large size of the plastid, which in turn is determined by the number of included starch grains (fig. 51).

At the metaphase the daughter plastids occupy their normal position at the respective poles of the cell. The spindle is not pointed at this stage as in cases previously described. Instead, the spindle poles are still relatively broad. This may be accounted for by the presence of large starch grains within the daughter plastids which inhibit the shortening and bending of the plastid and thus the formation of the pointed spindle (fig. 53).

During late anaphases the daughter chromosomes become appressed to the daughter plastids (fig. 55). At this stage there may be an abundance of starch grains in each daughter plastid. Although the presence of such large amounts of starch may delay the completion of the division of the plastid, it does not prevent the plastid halves from assuming polar positions (figs. 51, 53, 54).

With the completion of cell division the new plastid becomes appressed to the side of the nucleus away from the newly formed cell wall. The kidney shape of the nucleus resulting from the close association between nucleus and plastid commonly occurs in new daughter cells (fig. 56).

The behavior of plastids in maturing cells of the secondary parenchyma is reminiscent of their behavior in cells undergoing maturation in other tissues of the sporophyte. The maturing cells of the secondary parenchyma lie to the outside of the cambium and of the layer of meristematic cells it produces. The plastid in contact with the nucleus of a maturing cell divides, and its progeny in turn may undergo several divisions (fig. 57). The division of the plastid does not initiate nuclear division, nor do the daughter plastids show any tendency to occupy polar positions within the cell. In the older cells toward the periphery of the secondary parenchyma the starch grains already present within the plastids enlarge rapidly, so that the plastids containing them fill a major portion of the cell. As the starch grains enlarge, the plastid membranes disappear. In a mature storage cell of the secondary parenchyma there may be thirteen to twenty nonlaminated starch grains (fig. 58).

Function of plastids

The most apparent function of the leucoplast in the mature cells of the sporophyte is the storage of starch. Starch is first visible as a thin violet-staining layer enveloping some of the grana in the plastid (figs. 28, 32–34). The grana represent centers about which the accumulation of starch occurs, and, as the starch grains increase in size, the position occupied by each of the grana becomes the position of the refractive hilum of the mature starch grain.

Starch is not formed about all the grana of the leucoplast. Those that have no starch associated with them are usually located at the periphery of the plastid. As the spindle is formed between the inner surfaces of the daughter plastids and the nuclear membrane, the spindle fibers become localized in groups lying adjacent to the peripheral grana of the lower surface of the plastid (figs. 13, 14, 53, 59). By late anaphase the fibers associated with the inner surfaces of the daughter plastids have disappeared, giving way to dark-staining granular areas (figs. 15, 43).

Whether the grana function in the formation of the spindle fibers was not determined; it is clear, however, that spindle

formation is dependent upon the presence of the plastids. In every meristematic cell observed whose nucleus was at late prophase, the newly formed spindle terminated at the inner surface of the daughter plastids (figs. 13, 40, 41, 52). The importance of the plastid in spindle formation is emphasized by the anomalous behavior of the plastids in some dividing cells. One cambial initial whose nucleus was in a prophase contained three instead of the usual two daughter plastids (fig. 59), and the spindle formed was tripolar, each pole being located on the inner surface of one of the three plastids.

The chloroplasts also, like the leucoplasts, function in starch formation. The starch formed by the chloroplast is stored temporarily *in situ*. The presence of starch in the chloroplasts may be demonstrated by flooding free-hand sections of leaves with Graham's iodine. The starch is shown to have been deposited about some of the grana in the chloroplast.

The distribution of the photosynthetic pigments of the chloroplast was not determined with certainty. YUASA (**27**) suggested that the chlorophyll in the chloroplast of *Selaginella* is associated with the network of grana and connecting threads. This network in the chloroplast of *I. muricata* var. *braunii* is opaque, and it is therefore impossible to determine whether YUASA's statement holds for this species.

Discussion

STRUCTURE OF CHLOROPLAST.—The structure of the chloroplast of *I. muricata* var. *braunii*, as studied *in vivo* and in killed and stained preparations, is a combination of the structure described by MEYER (**17**) for the chloroplast of *Acanthophippium* and by YUASA (**26, 27**) for that of *Selaginella*. MEYER observed a peripheral distribution of linearly arranged, disk-shaped grana. YUASA figured connecting fibers between spindle-shaped grana which lie well within the stroma of the chloroplast away from its periphery. The chloroplast of *I. muricata* var. *braunii* has spindle-shaped grana linearly arranged. Between them are connecting threads forming a network within the chloroplast. This network is distributed throughout the body of the plastid—neither entirely peripheral as seen by MEYER in *Acanthophippium* nor entirely internal as described by YUASA.

MCALLISTER (**14, 15**) and MA (**10, 11**) have considered the red-staining spindle-shaped grana of the chloroplasts of *Anthoceros*, *Isoetes*, and *Selaginella* as pyrenoids, comparable with those of the green algae. They described the "pyrenoids" as having a length of from 0.5 to 2.0 μ. The average length of the spindle-shaped grana in the chloroplasts of *I. muricata* var. *braunii* is 0.85 μ—well within the size range of the "pyrenoids" as described by MCALLISTER and MA. On the basis of size and shape their "pyrenoids" thus seem to be the same structures described as grana in the chloroplasts of *I. muricata* var. *braunii*. Further supporting evidence is YUASA's (**27**) description of the spindle-shaped grana of *Selaginella* as having a length of from 0.5 μ to 2 μ.

DUNLOP (**4**) also observed red-staining granules in the chloroplasts of *Selaginella*, but his preparations gave no indication that these granules functioned in the formation of starch. The red-staining granules which the writer has observed in the chloroplasts of *I. macrospora* and *I. muricata* var. *braunii* are highly refractive, and each is surrounded by a layer of violet-staining starch. It would seem

that they represent the hila of the young starch grains.

STRUCTURE OF LEUCOPLASTS.—There are very few accounts of plastid structure in the meristematic cells of plants belonging to the Lycopsida. MARQUETTE (13) mentioned the presence of delicate fibers extending between the starch grains and the periphery of the plastid. DUNLOP (4) reported red-staining granules in the plastids of the root tip cells of *Lycopodium*.

The internal structure of the leucoplasts of meristematic sporophytic cells of *I. macrospora* and *I. muricata* var. *braunii*, like that of chloroplasts, includes grana and connecting fibers forming a network through the body of the leucoplast. The network and stroma of the leucoplast obviously lack photosynthetic pigments; however, starch accumulates about the grana of the leucoplast as about those of the chloroplast. The starch stored by the leucoplast accumulates until large quantities of storage starch are present. This is in contrast to the temporary storage of starch by the chloroplast.

Starch formation in the plastids of spermatophytes and many pteridophytes is accomplished by the accumulation of starch either at the center or at the periphery of the plastid. This method of starch formation is essentially similar to the accumulation of starch around the grana in the plastids of *Isoetes*. It does not involve the transformation of the starch-forming body of the plastid into starch, as TIMBERLAKE (22), MCALLISTER (14, 15), and MA (10, 11, 12) have described for the pyrenoids of some green algae, bryophytes, and pteridophytes.

POLAR BEHAVIOR OF PLASTIDS.—MARQUETTE (13) was first to recognize the significance of the polar organization of cells as manifested by plastids. In 1928 MA confirmed MARQUETTE's observations when she described plastid polarity in the cells of the intercalary meristem of *I. melanopoda*. The most recent report of plastid polarity in *Isoetes* was by DUNLOP (4), who described the polar organization of the meristematic root tip cells of *I. muricata* var. *braunii*.

The only meristematic cells of *Isoetes* in which plastid polarity has previously been described are those of the root and leaf. The results of the present study indicate that all the meristematic cells of the mature sporophytes of *I. macrospora* and *I. muricata* var. *braunii* may exhibit plastid polarity.

It has been argued that plastid polarity may be without significance because the plastid of a maturing cell may divide, and the daughter plastids thus formed do not exhibit polarity. This argument does not take into consideration that the maturing cells which contain several plastids are in a more advanced stage of development. It is to be remembered that in many plants a uninucleate cell may by a series of divisions become multinucleate. This occurs only at a certain stage in the development of the cell or of the tissue of which the cell is a component.

ATKINSON (2) was of the opinion that plastid polarity as exhibited by the meristematic cells of the sporophyte of *Isoetes* may not indicate a polarity of the cell. She suggested that the polar positions assumed by the daughter plastids are a consequence of the large area occupied by the achromatic figure which makes it impossible for the daughter plastids to go elsewhere in the cell. The regularity that marks the sequence of plastid polarity seems to oppose this argument. In addition, large dividing meristematic cells with small spindle areas have been observed. Although there is ample opportunity in such cells for a ran-

dom location of the daughter plastids, these are nevertheless always at the poles of the spindle. Further evidence of the importance of cell polarity as evidenced by the plastids is in the formation of the spindle. In all division figures observed there is an intimate association between the spindle poles and the inner surfaces of the plastids.

Just as in the cases of centrosomes and blepharoplasts, the division of the single plastid in each meristematic cell of the *Isoetes* sporophyte precedes and seems to initiate the division of the nucleus, and the daughter plastids function in spindle formation. That the plastids are variable in form and function is borne out by their behavior in the cells of the mature sporophyte of *Isoetes*. Prior to division, the mother plastid of a meristematic cell becomes greatly elongated, so that each of the daughter plastids formed are nearly twice as long as the mother plastid of a cell in the metabolic phase. At the poles of the spindle each daughter plastid shortens and becomes crescent-shaped and in this way forms the pointed spindle so frequently observed. Finally, as the cell plate of the dividing cell is formed, the two daughter plastids assume the shape, size, and position of the original mother plastid.

A "resting" meristematic cell in each species studied normally contains only one plastid. One of two things may happen to the single plastid as the cell matures. If the maturing cell is destined to be a tracheid or some other nonliving element, the plastid disappears along with the rest of the living protoplast. If the mature cell remains alive, the single plastid may become filled with numerous starch grains, or it and its descendants may undergo several divisions, forming a variable number of plastids within the undivided cell. The daughter plastids thus formed will become either starch-filled leucoplasts of storage cells or chloroplasts of leaf cells. The division of the single plastid in such cases does not initiate nuclear division, nor do the daughter plastids formed show any tendency to occupy the poles of the cell. The plastids of such a maturing cell lose their intimate association with the nucleus and lie at random within the cell.

All the plastids in the cells of the mature sporophyte have been derived from fully formed pre-existing plastids and, here at least, is a structure of the cell which in its individuality resembles the nucleus. The question remains open whether this individuality is maintained not only from cell generation to cell generation through the life-history of the sporophyte but also through sporogenesis, the development of the gameophyte, and gametogenesis. Studies are already under way in an effort to provide the answers to these problems.

Summary

1. The visible structure of the chloroplasts of *Isoetes* is unaltered by the action of various killing agents.

2. The structure of chloroplast and leucoplast includes a network of grana and connecting fibers. The network is distributed through the stroma of the plastid.

3. The red-staining granules observed in the plastids of *Isoetes* are refractive hila of young starch grains.

4. The grana are the centers about which starch is deposited within the plastids.

5. There is only one plastid in each "resting" meristematic cell of the sporophyte.

6. Division of the mother plastid within such a meristematic cell begins while the nucleus is still in the metabolic phase.

The division of the plastid may initiate nuclear division.

7. The two daughter plastids thus formed function in the formation of the achromatic figure. The fibers of the achromatic figure terminate at the inner surface of each daughter plastid and appear to be attached to that surface.

8. The transition from the broad spindle poles at prophase to the pointed spindle poles at metaphase is brought about by a shortening and bending of the plastids at the spindle poles.

9. Plastids of meristematic cells are variable in form and function. In dividing cells the division of the plastid follows a definite sequence of changes in size, shape, and position until cell division is complete.

10. In maturing cells the plastid and its progeny may divide several times without indication of plastid polarity. The daughter plastids thus formed may metamorphose into the chloroplasts of the mature photosynthetic cell or the starch-containing leucoplasts of storage tissues.

11. Plastid polarity has been observed in all the meristematic regions of the sporophytes of *I. macrospora* and *I. muricata* var. *braunii*.

12. Plastids found in the cells of the mature sporophyte are derived from preexisting, fully formed plastids. The individuality of the plastid in these cells is at the same level as the individuality of the nucleus.

I wish to express my sincere appreciation to Professor C. E. ALLEN of the University of Wisconsin for the many helpful suggestions given in the course of this investigation.

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LITERATURE CITED

1. ALLEN, C. E. Four-lobed spore mother cells in *Catherinea*. Amer. Jour. Bot. **3**:456–460. 1916.
2. ATKINSON, LENETTE R. Cytology. Pp. 196–232 in Manual of Pteridology, ed. F. VERDOORN. The Hague. 1938.
3. DAVIS, B. M. The spore-mother-cell of *Anthoceros*. BOT. GAZ. **28**:89–109. 1899.
4. DUNLOP, D. W. Cytology of the Lycopsida. Unpublished thesis, Univ. Wisconsin. 1940.
5. EKAMBERAM, T., and VENKATANATHAN, T. N. Studies on *Isoetes coromandelina*. Jour. Ind. Bot. Soc. **12**:192–225. 1933.
6. FITTING, H. Bau und Entwickelungsgeschichte der Makrosporen von *Isoetes* und *Selaginella* und ihre Bedeutung für die Kenntniss des Wachstums pflanzlicher Zellmembranen. Bot. Zeit. **58**:109–163. 1900.
7. HABERLANDT, S. Die Chlorophyllkörper der Selaginellen. Flora **71**:291–308. 1888.
8. HEITZ, E. Untersuchungen über den Bau der Plastiden. Planta **26**:134–163. 1936.
9. LANDER, CAROLINE A. The relation of the plastid to nuclear division in *Anthoceros laevis*. Amer. Jour. Bot. **22**:42–51. 1935.
10. MA, ROBERTA M. The chloroplasts of *Isoetes melanopoda*. Amer. Jour. Bot. **15**:277–283. 1928.
11. ———. The chloroplasts of *Selaginella*. Bull. Torrey Bot. Club **57**:277–285. 1930.
12. ———. Starch deposition in sporogenous cells of certain mosses. Bull. Torrey Bot. Club **57**:525–533. 1931.
13. MARQUETTE, W. Manifestations of polarity in plant cells which apparently are without centrosomes. Beih. Bot. Centralbl. **21**:281–303. 1907.
14. MCALLISTER, F. The pyrenoid of *Anthoceros*. Amer. Jour. Bot. **1**:79–93. 1914.
15. ———. The pyrenoids of *Anthoceros* and *Notothylas* with especial reference to their presence in spore mother cells. Amer. Jour. Bot. **14**:246–256. 1926.
16. MELIN, E. Die Sporogenese von *Sphagnum squarrosum* Pers. nebst einigen Bemerkungen über das Antheridium von *Sphagnum acutifolium*. Svensk Bot. Tidskr. **9**:262–293. 1915.
17. MEYER, A. Das Chlorophyllkorn in chemischer, morphologischer und biologischer Beziehung. Leipzig. 1883.
18. NÄGELI, C. Zellkerne, Zellenbildung, und Zellenwachstum bei den Pflanzen. Zeitschr. wiss. Bot. **1**:34–118. 1844.
19. SAPĚHIN, A. A. Untersuchungen über die Individualität der Plastide. Arch. Zellf. **13**:319–398. 1915.
20. SCHIMPER, A. F. W. Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. Jahrb. wiss. Bot. **16**:1–247. 1885.

21. STRASBURGER, E. Zellbildung und Zellteilung. 3. Aufl. Jena. 1880.
22. TIMBERLAKE, H. G. Starch formation in *Hydrodictyon utriculatum*. Ann. Bot. 15:619–635. 1901.
23. WEIER, T. E. Viability of cells containing chloroplasts with an optically homogeneous or granular structure. Protoplasma 31:346–350. 1938.
24. ———. The structure of the chloroplast. Bot. Rev. 4:497–525. 1938.
25. ———. The microscopic appearance of the chloroplast. Protoplasma 32:145–152. 1939.
26. YUASA, A. Studies in the cytology of Pteridophyta 19. The structure of the chloroplasts of some pteridophytic plants, observed on fixed and stained material. Bot. Mag. Tokyo 54:215–222. 1940.
27. ———. Studies in the cytology of Pteridophyta 20. The process of the formation of starch grains in the chloroplasts. Bot. Mag. Tokyo 54:338–342. 1940.
28. ZIRKLE, C. The structure of the chloroplasts in certain higher plants. Part I. Amer. Jour. Bot. 13:301–320. 1926.

SOME USES OF ACTIVATED CARBON IN CONTRATOXIFICATION[1] OF PLANT GROWTH-REGULATORS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 602

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The value of activated carbon and other contratoxicants to eliminate partially or completely the toxic effects of plant growth-regulators on plants was recently demonstrated by the author in greenhouse trials at the University of Chicago (2). One purpose of the experiments reported in this paper was to repeat some of these tests in the field.

Herbicidal sprays of plant growth-regulators applied after planting but before emergence of seedling crops (pre-emergence spraying) are often impractical because the compounds often injure the seedlings. These applications are usually very injurious to germinating dicotyledonous plants and may also damage some varieties of monocotyledons. One objective of the experiments here reported was to devise methods by which germinating seeds could be protected from pre-emergence applications of plant growth-regulators.

[1] "Contratoxification" refers to the application of absorbents, adsorbents, or ion exchangers to soils or parts of plants in order to accomplish partial or complete elimination of the toxic effects of plant growth-regulators on plants.

Contratoxification in soil

A fertile garden soil was used at the University of Chicago. The field plots were 4 × 4 feet with 1-foot border rows. Plant growth-regulators were obtained from commercial sources. About 1 liter of an aqueous solution containing 1.67 gm. of one of the growth-regulators was uniformly applied with a 3-gallon sprayer to a plot, a rate equivalent to an application of 10 lb./acre. These experiments were performed from May to September, 1948.

CONTRATOXIFICATION OF ENTIRE AREA TREATED WITH GROWTH-REGULATOR

The purpose was to determine the quantity of Norit A required to contratoxify ammonium 2,4-dichlorophenoxyacetate [NH_4(2,4-D)] in a garden soil. On May 25 five plots were laid out, and four were treated with NH_4(2,4-D) at a rate of 10 lb./acre. All plots were then watered, and the following day they were raked to a depth of about 3 inches so that all large clods were broken up. Next, 333, 83, 16.7, or 0 gm. of Norit A

in dust form were uniformly added to separate plots previously treated with NH_4(2,4-D). These applications were equivalent to rates of about 2000, 500, 100, or 0 lb./acre. The contratoxicant was mixed with the upper 3 inches of soil by raking, and then the plots were watered. On May 27 forty seeds of Red Kidney bean, twenty of Illini soybeans, and twenty of Alaska peas were uniformly planted in each plot.

On June 9 emergence counts were made, and on July 15, 49 days after planting, fresh weights of tops were obtained (table 1, fig. 1). Norit A was very effective in eliminating the toxic effects of the growth-regulator in the soil. Plants in plots previously treated with NH_4(2,4-D) to which 2000 or 500 lb. of Norit A per acre had subsequently been added made normal growth. In these plots and in the untreated control plot the average heights of Red Kidney bean, soybean, and peas at time of harvest were 15, 18, and 12 inches, respectively, and the peas had produced pods. Kidney beans and soybeans were only 10 inches tall in the plot previously treated with NH_4(2,4-D) to which 100 lb. of Norit A per acre were also added. There were only a few stunted kidney beans and soybeans 5 inches tall in the plot which received NH_4(2,4-D) only. Thus an amount between 100 and 500 lb. of Norit A per acre would be required for complete contratoxification of the growth-regulator under conditions of this experiment.

TABLE 1

EMERGENCE COUNTS AND FRESH WEIGHT (GM.) OF RED KIDNEY BEAN, SOYBEAN, AND PEA FROM SOIL PREVIOUSLY TREATED WITH NH_4(2,4-D) AT RATE EQUIVALENT TO 10 LB./ACRE NORIT A ADDED TO SOIL 1 DAY BEFORE PLANTING

Treatment	Amount of Norit A used (lb./acre)	Bean		Soybean		Pea	
		No. emerged	Weight of tops	No. emerged	Weight of tops	No. emerged	Weight of tops
Control	0	30	518	15	81	20	28
Norit A and NH_4(2,4-D)	2000	29	387	13	58	20	32
	500	31	543	13	77	18	29
	100	21	285	0	15	0	0
	0	0	38	0	2	0	0

CONTRATOXIFICATION OF GROWTH-REGULATOR ABOVE SEEDS

The purpose was to determine if a cone of contratoxicant above the seeds would protect germinating seeds from applications of a growth-regulator to the soil surface and also to ascertain whether protection is obtained if a row of seeds is covered with contratoxicant. On May 26 four plots were staked out, and in half of each plot two furrows $2\frac{1}{2}$ inches wide, 3 inches deep, and 4 feet long were dug. In the remaining portion of each plot holes $2\frac{1}{2}$ inches deep, 2 inches in diameter at the soil surface, and 6 inches apart were made with a dibble. The holes were funnel-shaped, with the widest portion at the soil surface. Red Kidney beans, Spancross sweet corn, or Alaska peas were uniformly planted in the furrows and holes. Two seeds of each species were planted separately in each of six holes, and twelve of each type were planted in

the furrows. The seeds were covered with ordinary soil in two plots, and in the other two they were covered with contratoxicant, a mixture of ten parts soil to one part Norit A on a dry-weight basis. NH_4(2,4-D) was then applied to an average foliage height of 13 and 15 inches, respectively. Beans and peas in plots to which contratoxicant was added above the seeds grew normally whether or not NH_4(2,4-D) was subsequently added, but plants from seeds in plots

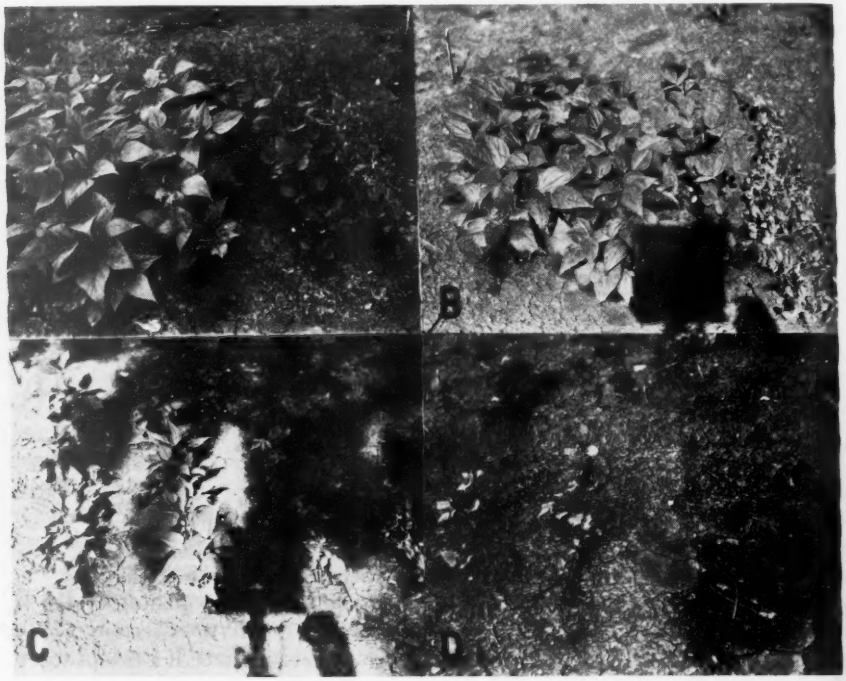

FIG. 1.—Growth of kidney beans, soybeans, and peas in soil treated with NH_4(2,4-D) at a rate equivalent to 10 lb./acre. Norit A added to soil 1 day before planting. *A*, untreated control; *B*, NH_4(2,4-D)—then 500 lb./acre of Norit A; *C*, NH_4(2,4-D)—then 100 lb./acre of Norit A; *D*, NH_4(2,4-D) only. Note that 100 lb./acre of Norit A resulted in partial and 500 lb./acre in complete contratoxification of growth-regulator.

one plot with and one without contratoxicant, after which all plots were watered.

Emergence counts on June 9, 14 days after planting, and fresh weights of tops on July 15 showed that the contratoxicant above the seeds gave excellent protection against NH_4(2,4-D) (table 2, fig. 2). At harvest time kidney beans and peas in the untreated control plot had unprotected by contratoxicant and treated with growth-regulator were much stunted. Although Spancross sweet corn was retarded in growth during its early development in plots treated with growth-regulator only, it was as large as the untreated control plants by harvest time.

In a second experiment holes $2\frac{1}{2}$ inches deep and 2 inches in diameter at the soil

surface were made with a dibble. The holes were about 6 inches apart. On June 10 seeds of Red Kidney bean, soybean, and pea were uniformly planted in separate holes, two seeds of one species being placed in each hole. In one plot the seeds were covered with contratoxicant, three parts soil to one part Norit A by weight, and in two other plots the seeds were covered with ordinary soil. One plot with and one without contratoxicant were then treated with $NH_4(2,4\text{-}D)$.

Emergence counts were made on June 28, 18 days after planting, and fresh weights were obtained on July 23 (table 3, fig. 3). The results were similar to

TABLE 2

NUMBER OF PLANTS WHICH EMERGED FROM PLOTS TREATED WITH $NH_4(2,4\text{-}D)$ SEEDS IN SOME PLOTS PROTECTED BY CONTRATOXICANT ABOVE SEEDS. FRESH WEIGHT (GM.) OF TOPS DETERMINED 50 DAYS AFTER PLANTING

	Kidney bean		Pea	
	No emerged	Weight of tops	No. emerged	Weight of tops
Untreated control	13	265	22	51
Norit A—no $NH_4(2,4\text{-}D)$	8	263	14	25
Norit A—then $NH_4(2,4\text{-}D)$	14	302	19	40
No Norit A—$NH_4(2,4\text{-}D)$	4	82	3	7

FIG. 2.—Growth of kidney bean, sweet corn, and pea from soil treated with $NH_4(2,4\text{-}D)$. Contratoxicant was applied above seeds in some plots. *A*, control; *B*, contratoxicant only; *C*, contratoxicant—then $NH_4(2,4\text{-}D)$; *D*, $NH_4(2,4\text{-}D)$ only. Note that presence of contratoxicant above seeds enabled plants to grow normally. Photo taken 29 days after planting.

those in the preceding experiment. The contratoxicant above the seeds gave much protection against the application of growth-regulator. The protected plants grew well, although not so vigorously as the untreated control plants. Many weeds of common purslane (*Portulaca oleracea* L.), milk purslane (*Euphorbia maculata* L.), and lamb's-quarters (*Chenopodium album* L.) grew in the control plot, but practically none grew in the plots treated with growth-regulator.

dinary soil was the untreated control; NH_4(2,4-D) was applied to all other plots.

Emergence counts on July 23, 7 days after planting, and fresh weight of tops on August 30 showed that a cone of contratoxicant $\frac{1}{2}$ inch in diameter furnished much protection against the growth-regulator, although a cone 1 inch in diameter furnished more (table 4).

This experiment was repeated using peas. The contratoxicant gave some

TABLE 3

EMERGENCE COUNTS AND FRESH WEIGHT (GM.) OF TOPS OF RED KIDNEY BEAN, SOYBEAN, AND PEA FROM SOIL TREATED WITH NH_4(2,4-D)*

	Kidney bean		Soybean		Pea		Weeds (weight of tops)
	No. emerged	Weight of tops	No. emerged	Weight of tops	No. emerged	Weight of tops	
Untreated control	23	333	17	96	20	13	168
Norit A—then NH_4(2,4-D)	22	259	18	33	19	11	2
No Norit A—NH_4(2,4-D)	2	4	0	0	0	0	2

* There was a cone of contratoxicant above seeds in one plot.

Size of contratoxified zone above seeds

The purpose was to determine how large the cone of contratoxicant above the seed must be in order to furnish protection against growth-regulators. On July 16 five plots were laid out, and forty holes about 6 inches apart and $2\frac{1}{2}$ inches deep were made in each plot with a dibble. The holes were $1\frac{1}{2}$ inches in diameter at the soil surface in three plots, 1 inch in diameter in another, and $\frac{1}{2}$ inch in the fifth. Two seeds of soybean were placed in each hole. The seeds in two plots with $1\frac{1}{2}$-inch holes were covered with ordinary soil, and the holes in the other plots were filled with contratoxicant, a mixture of ten parts soil to one part Norit A by weight. One plot in which the seeds were covered with or-

protection against the growth-regulator but less than that furnished the soybeans (table 4). This may be partially explained by the greater sensitivity of pea to NH_4(2,4-D).

Concentration of Norit A above seeds

The purpose was to determine the concentration of Norit A in a soil necessary to protect seeds from an application of growth-regulator. The effectiveness of sprinkling undiluted Norit A on the soil surface above the seed after planting was also studied. Holes $2\frac{1}{2}$ inches deep, 2 inches in diameter at the soil surface, and 6 inches apart were made. Two pea seeds were placed in each hole. Twenty-eight holes were filled with ordinary soil. Fourteen of them served as the untreated con-

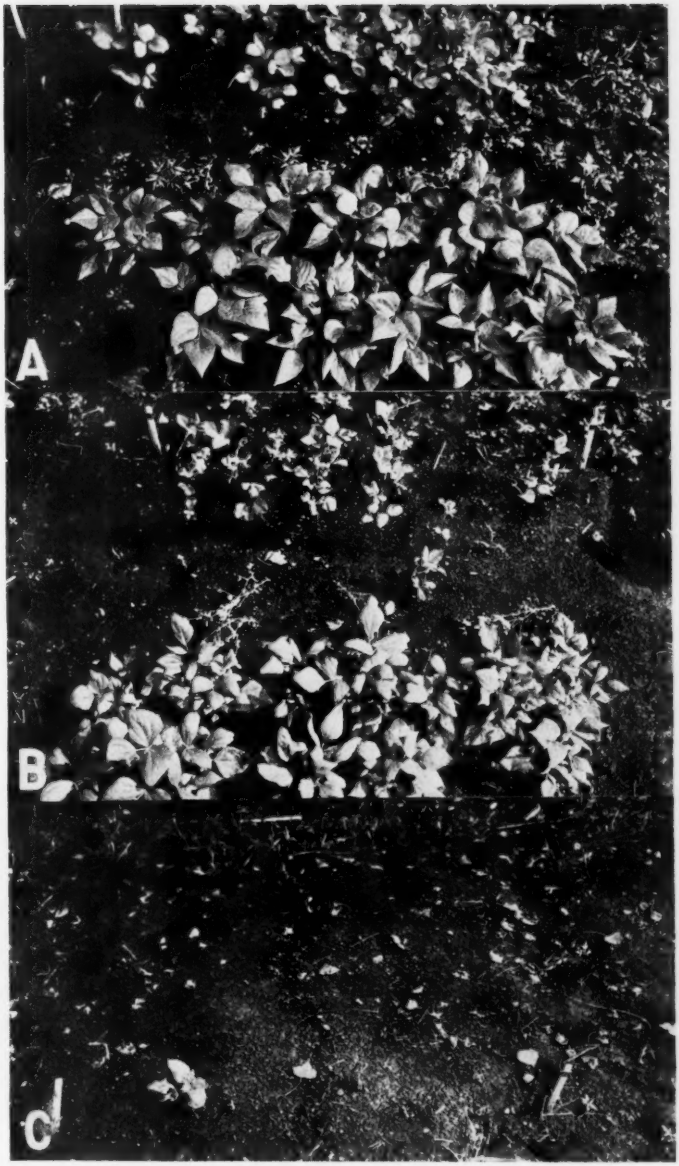

FIG. 3.—Growth of kidney beans, soybeans, and peas from soil treated with NH_4(2,4-D). Seeds in one plot covered with contratoxicant. *A*, control; *B*, contratoxicant—then NH_4(2,4-D); *C*, NH_4(2,4-D) only. Note that contratoxicant above seeds furnished much protection to plants. Many weeds grew in control plot but almost none in plots sprayed with growth-regulator. Photo taken 28 days after planting.

trol. Undiluted Norit A was sprinkled on the soil in a thin layer about 2 inches in diameter over the seeds in each of the other holes filled with ordinary soil. Other holes were filled with mixtures of Norit A in soil at concentrations of 333,333, 100,000, 20,000, 3333, 1000, 200, or 0 p.p.m. There were fourteen holes per treatment. The whole surface area, except the untreated control, was then treated with NH_4(2,4-D) at a rate of 10 lb./acre.

TABLE 4

NUMBER OF SOYBEANS AND PEAS THAT EMERGED AND FRESH WEIGHT (GM.) OF TOPS OF SOYBEANS FROM SOIL TREATED WITH NH_4(2,4-D). SEEDS IN SOME PLOTS PROTECTED BY CONE OF CONTRATOXICANT OF 1½, 1, OR ½ INCH DIAMETER; EMERGENCE COUNTS MADE 7 AND 13 DAYS AFTER PLANTING, RESPECTIVELY

Treatment	Soybean		Pea
	No. emerged	Weight of tops	No. emerged
Untreated control	57	230	40
Cone of contratoxicant 1½ inch in diameter	43	109	12
Cone of contratoxicant 1 inch in diameter	45	113	11
Cone of contratoxicant ½ inch in diameter	50	52	5
No contratoxicant	10	1	1

Growth of the peas was very poor and irregular because of the high temperatures prevailing. Emergence counts on June 28, 18 days after planting, and fresh weights of tops on July 15 showed that a concentration as low as 1000 p.p.m. of Norit A gave much protection to the plants. The undiluted Norit A sprinkled on the soil surface above the seeds also furnished much protection.

Adsorptive power of contratoxicant for growth-regulator after mixing with soil

The purpose was to determine how long Norit A incorporated in a soil retains its adsorptive capacity for a growth-regulator. It may be that the contratoxicant would adsorb nutrients and other compounds from the soil and become saturated, thus losing its power to adsorb growth-regulators. This is of importance, since the spraying of herbicidal growth-regulators is often done several days after crops are planted although before their emergence. If Norit A above seeds loses its adsorptive power for a growth-regulator between planting time and application of herbicide, the contratoxicant would be of no value.

On June 25, thirty-six holes 2 inches deep and 1½ inches in diameter at the soil surface were uniformly made in each of four plots. Two pea seeds were placed in each hole. The seeds were covered with ordinary soil in two plots and in the other two plots with contratoxicant, a mixture of one part Norit A to ten parts soil by weight. One plot with and one without contratoxicant were immediately treated with NH_4(2,4-D). All plots were watered, and watering was repeated daily for the duration of the experiment in order to allow nutrients and other compounds in the soil solution to diffuse to the contratoxicant. Three days after planting, a plot with contratoxicant was treated with growth-regulator.

Emergence counts on July 8, 13 days after planting, showed that the contratoxicant was effective in adsorbing growth-regulator for a period of at least 3 days after being incorporated in the soil (table 5). Fresh weights of tops were not obtained, since the peas did not grow well during the high temperatures of July.

TABLE 5

NUMBER OF PEAS THAT EMERGED FROM PLOTS TREATED WITH NH_4(2,4-D) 0 OR 3 DAYS AFTER PLANTING. SEEDS IN SOME PLOTS PROTECTED BY CONTRATOXICANT ABOVE THEM

Treatment	No. emerged
Untreated control	40
No contratoxicant—NH_4(2,4-D)	1
Contratoxicant—NH_4(2,4-D) applied immediately after planting	28
Contratoxicant—NH_4(2,4-D) applied 3 days after planting	38

In a second experiment Norit A was raked with the upper 2 inches of soil of plots at a rate equivalent to 500 lb./acre. Immediately afterward one plot with and one without Norit A received an application of NH_4(2,4-D) at a rate of 10 lb./acre. Other plots were treated with NH_4(2,4-D) 2, 4, 8, or 12 days after Norit A was applied. Two days after application of the growth-regulator twenty-five seeds of Red Kidney bean and twenty-five of soybean were uniformly planted in each plot. Seeds were also planted in untreated control plots and in plots which were treated only with growth-regulator 0 or 8 days after the initiation of the experiment. All plots were watered daily.

Emergence counts 3 weeks after planting indicated that Norit A still strongly adsorbed NH_4(2,4-D) even after the contratoxicant had been incorporated within the soil for 12 days (table 6).

COATING SEEDS WITH CONTRATOXICANT

The purpose was to determine whether coating seeds with Norit A before planting protects them from applications of growth-regulator after planting. On July 24 two plots were treated with NH_4-(2,4-D). The soil was then raked to a depth of 2 inches, and in one of the plots with and in another without growth-regulator one hundred seeds of Red Kidney bean were uniformly planted. In the other plot treated with growth-regulator, kidney beans coated with Norit A by immersion in an aqueous paste of the contratoxicant were planted. All plots were then watered.

Emergence counts on August 9, 16 days after planting, and fresh weights of

TABLE 6

NUMBER OF KIDNEY BEANS AND SOYBEANS THAT EMERGED FROM SOILS TREATED WITH NORIT A AND/OR NH_4(2,4-D)

Time of application of growth-regulator after addition of Norit A (days)	Bean			Soybean		
	Control	Norit A—then NH_4(2,4-D)	NH_4(2,4-D)	Control	Norit A—then NH_4(2,4-D)	NH_4(2,4-D)
0	21	20	11	20	17	1
2	.....	18	.....	.....	19	.....
4	.....	20	.....	.....	11	.....
8	21	20	2	17	12	0
12	.....	21	.....	.....	13	.....

tops on August 31 indicated that the contratoxicant furnished some protection (table 7). This experiment was repeated twice with similar results. Coating seeds of soybean, pea, or wheat with Norit A, however, did not furnish them protection.

TABLE 7

EMERGENCE COUNTS AND FRESH WEIGHTS (GM.) OF TOPS OF RED KIDNEY BEAN. SOME SEEDS COATED WITH NORIT A BEFORE PLANTING

	No. emerged	Weight of tops
Control	77	1000.2
Norit A on seeds	52	233.5
No Norit A on seeds	0	14.0

Contratoxification on aerial parts of plants

Red Kidney beans, soybeans, and peas were planted in 6-foot rows in the garden. Plant growth-regulators and suspensions of contratoxicant were sprayed with 3-gallon sprayers. The leaves were thoroughly wet with suspensions of contratoxicants. The plants were sprayed heavily with 0.1% solutions of growth-regulators but not to the extent that liquid dripped from the leaves. Dusting of contratoxicants was done with a cheesecloth bag. Each treatment consisted of one 3-foot row of plants containing about twenty plants of kidney bean, twenty-five of soybean, or twenty-five of peas.

VARIOUS RATES OF NORIT A

The purpose was to determine the quantity of Norit A in water necessary to protect kidney beans against NH_4-(2,4-D). On June 20, a sunny day, the average height of the plants was 8 inches and they had expanded two trifoliate leaves. At 10:00 A.M., plants were sprayed with 20, 5, 1, 0.33, or 0% aqueous suspensions of Norit A. Ten minutes later some plots treated with contratoxicant were sprayed with a 0.1% solution of NH_4(2,4-D). One lot of plants received an application of contratoxicant only; another was untreated and served as a control.

Plants were harvested on July 15, 25 days after treatment (table 8, fig. 4). Control plants and those treated with contratoxicant only were about 13 inches tall, and those sprayed only with NH_4(2,4-D) were dead and dried. The 20% and 5% suspensions of contratoxicant gave excellent protection against the growth-regulator, the 1% gave much, but the 0.33% suspension gave very little protection. Plants sprayed only with 20% or 5% suspensions of Norit A produced less fresh weight of tops than the untreated control plants.

USE OF NORIT A DUST

The purpose was to determine whether Norit A dust protects soybeans against applications of NH_4(2,4-D), sodium 2,4-dichlorophenoxyacetate [Na(2,4-D)], or isopropyl ester of 2,4-dichlorophenoxyacetic acid (IE 2,4-D). At the time of treatment, 11:00 A.M., June 24, the plants were 9 inches tall, and the third trifoliate leaves were beginning to ex-

TABLE 8

FRESH WEIGHT OF TOPS (GM.) OF KIDNEY BEANS 25 DAYS AFTER TREATMENT WITH NORIT A AND/OR 0.1% NH_4(2,4-D)

Treatment	With NH_4(2,4-D)	Without NH_4(2,4-D)
20% Norit A	295	310
5% Norit A	335	308
1% Norit A	182	481
0.33% Norit A	90	413
No Norit A	12*	526

* Dead and dried.

FIG. 4.—Kidney beans 4 (*upper*), 16 (*middle*), and 25 (*lower*) days after spraying with aqueous suspensions of Norit A and/or growth-regulator. Rows (*left to right*): NH_4(2,4-D) only; 0.33% Norit A—then NH_4(2,4-D); 1% Norit A—then NH_4(2,4-D); 5% Norit A—then NH_4(2,4-D); and 20% Norit A—then NH_4(2,4-D). Note that 20% and 5% suspensions of Norit A gave excellent protection against growth-regulator and that 1% gave partial protection.

tops on August 31 indicated that the contratoxicant furnished some protection (table 7). This experiment was repeated twice with similar results. Coating seeds of soybean, pea, or wheat with Norit A, however, did not furnish them protection.

TABLE 7

EMERGENCE COUNTS AND FRESH WEIGHTS (GM.) OF TOPS OF RED KIDNEY BEAN. SOME SEEDS COATED WITH NORIT A BEFORE PLANTING

	No. emerged	Weight of tops
Control	77	1000.2
Norit A on seeds	52	233.5
No Norit A on seeds	0	14.0

Contratoxification on aerial parts of plants

Red Kidney beans, soybeans, and peas were planted in 6-foot rows in the garden. Plant growth-regulators and suspensions of contratoxicant were sprayed with 3-gallon sprayers. The leaves were thoroughly wet with suspensions of contratoxicants. The plants were sprayed heavily with 0.1% solutions of growth-regulators but not to the extent that liquid dripped from the leaves. Dusting of contratoxicants was done with a cheesecloth bag. Each treatment consisted of one 3-foot row of plants containing about twenty plants of kidney bean, twenty-five of soybean, or twenty-five of peas.

VARIOUS RATES OF NORIT A

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Plants were harvested on July 15, 25 days after treatment (table 8, fig. 4). Control plants and those treated with contratoxicant only were about 13 inches tall, and those sprayed only with NH_4(2,4-D) were dead and dried. The 20% and 5% suspensions of contratoxicant gave excellent protection against the growth-regulator, the 1% gave much, but the 0.33% suspension gave very little protection. Plants sprayed only with 20% or 5% suspensions of Norit A produced less fresh weight of tops than the untreated control plants.

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pand. Some plots were dusted with Norit A. Then, some plots treated with Norit A and some without previous treatment were sprayed with 0.1% aqueous solutions of Na(2,4-D), NH_4-(2,4-D), or IE 2,4-D. One plot received a treatment of contratoxicant only. A heavy rain falling $3\frac{1}{2}$ hours after treatment washed most of the contratoxicant from the foliage.

TABLE 9

FRESH WEIGHT (GM.) OF TOPS OF SOYBEAN 26 DAYS AFTER TREATMENT

Treatment	With Norit A	Without Norit A
Control	260	*
Na(2,4-D)	238	63†
NH_4(2,4-D)	192	43†
IE 2,4-D	147	14‡

* Sample lost.
† Plants alive but little developed.
‡ Dead and dried.

The plants were harvested on July 20, 26 days after treatment, when controls were 21 inches tall and beginning to flower (table 9). Plants sprayed with growth-regulators only were dead or almost dead, but the Norit A gave excellent protection against each growth-regulator used. Some leaves of plants dusted with Norit A and then sprayed with a growth-regulator were abnormally veined and appeared blistered, but the over-all growth was not severely retarded.

USE OF DUSTS OF NORIT A, NUCHAR 2A, AND NUCHAR C-115

The purpose was to compare the effectiveness of two types of Nuchar with Norit A in protection of soybeans from sprays of butyl ester of 2,4-dichlorophenoxyacetic acid (BE 2,4–D). The plants were treated when they were 9 inches tall with three trifoliate leaves. Plots were dusted with Norit A, Nuchar 2A, or Nuchar C-115. Some plots previously dusted and some not dusted with contratoxicant were sprayed with 0.1% BE 2,4-D. Some lots of plants received applications of contratoxicant only, and another received BE 2,4-D only. One untreated lot served as a control.

Final observations and harvest on July 26 showed that all contratoxicants gave excellent protection against the growth-regulator (table 10).

APPLICATION OF CONTRATOXICANTS AFTER SPRAYING WITH GROWTH-REGULATOR

The purpose was to determine whether Norit A could prevent toxicity symptoms of BE 2,4-D when applied to plants previously treated with BE 2,4-D. Kidney beans 8 inches tall with two trifoliate leaves and peas 10 inches high were used. At 7:00 A.M. on June 22, an overcast day, plants were sprayed with a 0.1% solution of BE 2,4-D. After 5, 15, and 60 minutes, or 5 hours peas were sprayed with a 5% aqueous suspension of Norit A. Beans were sprayed with contratoxicant 5, 15 minutes, or 3 hours after treatment with growth-regulator.

TABLE 10

FRESH WEIGHT (GM.) OF TOPS OF SOYBEAN 26 DAYS AFTER TREATMENT WITH CONTRATOXICANTS AND/OR GROWTH-REGULATOR

Treatment	With BE 2,4-D	Without BE 2,4-D
No contratoxicant	6*	260
Norit A	189	239
Nuchar 2A	223	244
Nuchar C-115	233	225

* Dead and dried.

Plants were harvested on July 8, 16 days after treatment, when the control

plants of peas were 14 inches tall and fruiting, and bean controls were 13 inches high (table 11). Peas sprayed with BE 2,4-D only and with BE 2,4-D and then contratoxicant after 5 hours were dead and dried. Peas treated with growth-regulator and then contratoxicant after 5 minutes were similar to the control plants, but those sprayed with contratoxicant after 15 minutes showed injury. The latter plants had swollen

TABLE 11

FRESH WEIGHT (GM.) OF TOPS OF KIDNEY BEANS AND PEAS TREATED WITH NORIT A AT VARYING INTERVALS AFTER SPRAYING WITH BE 2,4-D. SIXTEEN DAYS AFTER TREATMENT

Time of application of contratoxicant after spraying with BE 2,4-D	Peas	Bean
Untreated control	140	526
5 min.	122	225
15 min.	66	175
60 min.	50	
3 hr.		45†
5 hr.	16*	
No contratoxicant	13*	74†

* Dead and dried.
† Some plants alive but little developed.

stems, produced no fruit, and were beginning to die. Peas sprayed with contratoxicant after 60 minutes were almost dead.

The results for beans were similar to those for peas. Unless Norit A was applied within 15 minutes after treatment with BE 2,4-D, very little protection was afforded (table 11).

Discussion

Coating seeds of Red Kidney bean with contratoxicant gave some protection to germinating plants against a growth-regulator, but coating seeds of pea, soybean, or wheat gave none. The germinating Red Kidney bean probably distributed contratoxicant through the soil as the cotyledons were pushed upward and thereby made a contratoxified zone below the cotyledons. Mixing of contratoxicant with soil probably would not result with plants such as pea, the cotyledons of which fail to emerge from the soil. Roots and stems emerging from seeds coated with contratoxicant probably soon come in contact with soil containing toxic amounts of growth-regulator. A method enabling the contratoxicant on the seed coat to move outward with and thus protect growing roots and stems would be of value. A mucilaginous base incorporated with the contratoxicant might result in movement of the contratoxicant.

Coating of reproductive plant parts other than seeds with contratoxicants would probably furnish protection against growth-regulators. ARLE *et al.* (1) have pointed out that the dusting of sweet-potato slips with activated carbon before planting minimized injury to the sprouts from 2,4-D.

In these experiments, the excessive rate of 10 lb. of growth-regulator per acre was applied to the soil. It is probable that greater protection to germinating seedlings would have been obtained if lesser amounts of growth-regulator had been applied.

Summary

1. An amount between 100 and 500 lb. of Norit A per acre was required for complete contratoxification of ammonium 2,4-dichlorophenoxyacetic acid [NH_4-(2,4-D)] previously applied to a field soil at the rate of 10 lb./acre.

2. A cone of a Norit A—soil mixture above seeds, or such a mixture covering a row of seeds, protected the germinating

plants from broadcast applications of plant growth-regulators. Effective weed control was obtained in these plots.

3. A cone of contratoxicant $\frac{1}{2}$ inch in diameter at the soil surface above a seed gave much protection to the germinating seed against a plant growth-regulator later applied at the surface. A concentration of 1000 p.p.m. of Norit A in soil was sufficient to furnish much protection.

4. When Norit A was mixed with a field soil, it retained its adsorptive capacity for NH_4(2,4-D) for at least 12 days.

5. Coating seeds of Red Kidney bean with Norit A before planting in soil previously treated with NH_4(2,4-D) furnished partial protection to the germinating seeds.

6. A 5% aqueous suspension of Norit A sprayed on young Red Kidney bean plants protected them from a subsequent application of growth-regulator.

7. Norit A dusted on young soybeans protected the plants against subsequent applications of NH_4(2,4-D), sodium 2, 4-dichlorophenoxyacetate, or isopropyl ester of 2,4-dichlorophenoxyacetic acid. Dust applications of Norit A, Nuchar 2A, or Nuchar C-115 were effective in protecting plants against subsequent applications of growth-regulator.

8. Unless Norit A was applied within 15 minutes after plants were sprayed with growth-regulator, little protection was afforded.

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LITERATURE CITED

1. ARLE, H. F.; LEONARD, O. A.; and HARRIS, V. C. Inactivation of 2,4-D on sweet-potato slips with activated carbon. Science **107**:247–248. 1948.

2. WEAVER, R. J. Contratoxification of plant growth-regulators in soils and on plants. *Bot. Gaz.* **109**:276–300. 1948.

SOME EXPERIMENTS WITH HERBICIDES IN PINEAPPLES

A. S. CRAFTS[1] AND A. EMANUELLI

Introduction

One of the major items in the cost of producing pineapples in Puerto Rico is that of weeding. In most plantings there is an alternation of two or three rows planted closely and wide alleys (1). Whereas machine tillage can be used in the alleys, all weeding between plants in the close-planted double or treble rows must be done by hand.

Recent experiments with single-row planting prove that, although fewer plants can be produced per acre, the waste of land is more than compensated for by the savings in labor, because machine tillage can be used right up to the row. By attaching guards to a Ford-Ferguson tractor, all the land except a narrow strip in each plant row may be stirred by tillage instruments. This makes for very economical weed control.

When viewed from a long-range standpoint, considering utilization of soil nutrients and the evils of soil erosion, it seems that chemical weed control may offer real advantages over tillage. Observations have shown that frequent tillage has led to serious sheet erosion in Puerto Rican pineapple plantings. That the destruction of surface-feeding roots

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by tillage may also result in appreciable losses of soil nutrients because of rapid leaching through the root zone also seems probable. Destruction of weeds by chemical sprays would minimize erosion and permit development of surface-feeding pineapple roots that would absorb soil nutrients rapidly and efficiently.

The literature on weed control contains little information concerning practices in pineapple culture. Paper mulch has been used in Hawaii (**1, 6**), and various herbicides have been tried on a small scale in Puerto Rico, but none of these methods has been widely accepted. Care has been taken to avoid use of arsenicals around a crop that is used directly for human food.

Recent development of selective oil (**3, 5**) and dinitro sprays (**2**) indicated the possibility of using a weed-killer in pineapples that would not injure the plants. This paper describes some greenhouse and field trials that will aid in developing a weed-control program in this important crop.

Material and methods

Number 10 cans were perforated at the bottom, filled with a medium-textured greenhouse soil, and planted with shoots of the pineapple variety Red Spanish and with shoots or cuttings of a group of common Puerto Rican weeds. Some cultures of grasses were grown from seeds, and sensitive plants (*Mimosa pudica*) were transplanted from the field.

When the pineapple plants had set roots and were starting active growth, lots of plants selected for uniformity in size were sprayed, using a Smith "streamline blizzard continuous" hand sprayer. In some experiments the plants were sprayed until visibly wet; in others definite volumes of liquid were used, the covering being made as uniformly as possible. Most of the sprays wet the inside (ventral) surfaces of the leaves readily and were observed to collect and run down into the crowns. This was true of both oil and water sprays and also of emulsions. The outside (dorsal) surfaces proved very difficult to wet, and only the oil sprays covered these at all well.

Visual estimates of damage to tissues were made at intervals after spraying. The amount of injury, as expressed in the tables, is calculated as a percentage of the vegetation present at the time of application. Comparisons of such observations show clearly the trends of the action of the various chemicals.

Field trials were made using knapsack sprayers, and observations were completed within 10 days after application.

The first tests involved spraying with a group of commercial oil fractions.[2] These were stove naphtha, a light fraction used as a fuel in gasoline stoves; "Varsol," a slightly heavier fraction that conforms to the specifications of Stoddard solvent; a standard kerosene; a light diesel-oil fraction obtained by fractional distillation of the standard automotive diesel oil available in Puerto Rico into two equal fractions; standard diesel oil; heavy diesel, the other half of the distilled oil mentioned above; "Bayol 16," a flushing oil; and "Bunker C," a heavy black furnace fuel.

The plants used were pineapple, Bermuda grass (*Cynodon dactylon*), purslane (*Portulaca oleracea*), dayflower (*Commelina* spp.), nutgrass (*Cyperus rotundus*), sensitive plant (*Mimosa pudica*), bindweed (*Ipomoea* spp.), and tropical kudzu (*Pueraria hirsuta*), a tropical legume that resembles a number of weedy species and also serves as a broad-leaved crop plant.

[2] Samples kindly donated by the Standard Oil Co. of Puerto Rico.

Results

The results indicate that the only oils producing practically no injury on pineapple plants were stove naphtha and "Bunker C" (table 1). The first is a very light, volatile oil which evaporates almost as rapidly as it is applied. "Bunker C" is a heavy black oil and, although it coated the leaves, caused little or no direct injury. The coated leaves continued to grow at their bases, so that after several weeks only their tips were coated. Injury which followed the application of the oils of the medium range consisted of death of the meristematic tissues at the bases of the young growing leaves, decay of the tissues accompanied by a putrid odor, and complete loss of the centers of the plants. The older leaves in which no active meristem was left withstood the toxic action but later became chlorotic and died after several months. Figure 1 illustrates the effects of the diesel-oil spray and of the "Bunker C" treatment.

Injury to Bermuda grass by the light oils was rapid and, in these pot cultures, fatal (table 2). In deeper soil the roots would have survived. Injury by "Bayol 16" appeared on September 5. It was typical chronic injury,[3] evidenced by chlorosis of the leaves that gradually became severe enough to result in death by October 9. The stems did not die until some time after the leaves.

Injury to purslane by the light oils was rapid. Chronic injury developed in the cultures treated with the heavy oils. Since the plants had a long period in which to recover, those that were not killed recovered completely. Final readings were erratic and relate to the relative completeness of coverage more than to the characteristics of the oils.

Injury to dayflower was severe from the light and medium oils but slow and incomplete from the two heavy fractions ("Bayol 16" and "Bunker C"). Plants treated with the latter two showed considerable recovery toward the end of the period of observation, as did the plants in the culture treated with standard diesel oil.

Injury to nutgrass by these oils never exceeded 25%. As the older injured leaves died, new ones developed; recovery was complete by the end of the test. Sensitive plants were the most suscep-

TABLE 1

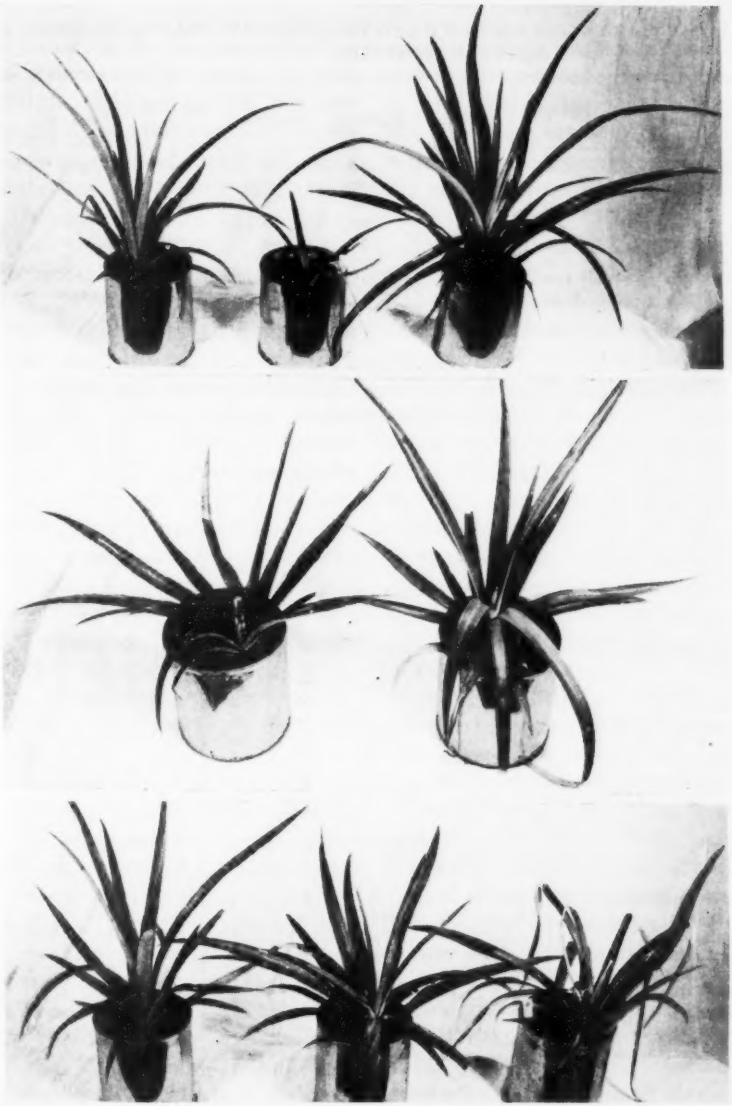
INJURY (%) TO PINEAPPLE CAUSED BY OIL SPRAYS. SPRAYED SEPTEMBER 3, 1947

OIL FRACTION	DATE OBSERVED								
	9/3*	9/10	9/16	9/18	9/20	9/23	9/27	10/9	10/18
Stove naphtha	0	0	0	0	0	0	0	0	0
"Varsol"	0	0	0	0	0	15	15	15	25†
Kerosene	0	50†	75	80	80	90	95	95	100
Light diesel	0	25†	75	80	80	90	90	100	100
Standard diesel	0	5	75†	80	80	90	90	100	100
Heavy diesel	0	10	10	20	50†	60	60	75	80
"Bayol 16"	0	0	75†	80	80	80	90	90	90
"Bunker C"	0	0	5	10	10	15	0	0	0

* Readings made on September 4 and 5 showed no injury to pineapple.

† Severe injury occurred in the center of the plant, resulting in death of the center bud and of all inner leaves.

[3] For detailed definition of this term as here used see CRAFTS and REIBER (5).



FIGS. 1–3.—Effect of herbicides on pineapple plants. Fig. 1 (*above*), oil sprays. *Left*, control plant; *center*, sprayed with diesel oil; *right*, sprayed with "Bunker C." Note on latter that older leaves are completely coated with black oil and that younger leaves are coated from tips down to portions that were in crown at time of spraying. Fig. 2 (*middle*), "Varsol." *Left*, plant received 5 ml. of "Varsol" on September 16. *Right*, control with leaves depressed to show center of crown. Photographed October 24. Fig. 3 (*below*), general contact sprays. *Left*, control; *center*, recently sprayed plant with front leaves depressed to show injury to bases of young leaves; *right*, sprayed plant that has had time for growth of injured leaves. Older leaves at time of spraying were uninjured; young leaves were injured in meristematic region but have since grown out, and only tips are dead.

TABLE 2

INJURY (%) TO WEEDS AND CROP PLANTS FROM OIL SPRAYS. TREATED SEPTEMBER 3, 1947. FINAL OBSERVATIONS ON OCTOBER 18

Oil fraction	Bermuda grass	Purslane	Dayflower	Nutgrass	Sensitive plant	Bindweed	Tropical kudzu
Stove naphtha	100	0	80	0	100	100	100
"Varsol"	100	25	100	0	100	0	0
Kerosene	0	100	100	0	100	0	0
Light diesel	0	100	100	0	100	0	0
Standard diesel	0	0	50	0	100	0	0
Heavy diesel	0	0	100	0	100	0	100
"Bayol 16"	100	0	25	0	100	0	0
"Bunker C"	10	20	15	0	0	100	0

TABLE 3

INJURY (%) TO PINEAPPLE PLANTS ON INDICATED DATES FROM OIL SPRAYS APPLIED SEPTEMBER 16, 1947

Oil fraction	Volume applied (ml.)							
	5	10	15	20	5	10	15	20
	September 18				September 20			
Stove naphtha	0	0	0	0	0	0	0	5
"Varsol"	0	0	10	25	0	50*	65*	75*
Kerosene	0	0	5	20	0	50*	65*	75*
Light diesel	0	0	0	0	0	50*	60*	70*
Standard diesel	0	0	0	0	0	50*	60*	70*
Heavy diesel	0	0	0	0	0	0	0	0
"Bayol 16"	0	0	0	0	0	0	0	0
"Navy Special"	0	0	0	0	0	0	0	0
	September 22				September 27			
Stove naphtha	0	5	0	0	0	5	0	0
"Varsol"	25	50	65	75	30	60	75	90
Kerosene	35	50	65	75	50*	75	95	90
Light diesel	50*	60	65	75	60	80	90	90
Standard diesel	0	60	65	75	50*	65	80	75
Heavy diesel	25	40	60*	75*	65*	90*	80	90
"Bayol 16"	0	0	0	0	0	0	0	50*
"Navy Special"	0	0	0	0	0	0	5	10
	October 9				October 18			
Stove naphtha	0	0	0	0	0	0	0	0
"Varsol"	20	60	95	95	25	75	95	95
Kerosene	60	85	100	100	50	90	100	100
Light diesel	70	100	100	100	75	100	100	100
Standard diesel	90	75	100	90	90	90	100	90
Heavy diesel	75	100	90	100	90	100	100	100
"Bayol 16"	0	70*	50*	100	5	70	70	100
"Navy Special"	0	5	10	15	0	5	10	15

* Severe injury occurred in the center of the plant, resulting in death of the center bud and all inner leaves.

tible of all to the oils and were killed by all but "Bunker C." Leaves sprayed with this oil remained coated, but new ones emerged, and the plants recovered.

Bindweed was killed rapidly by stove naphtha and did not recover. In the field, where the root system is deeper, injury would not have been so complete. Though all the light fractions caused killing of leaves, recovery by axillary buds and by new shoots from below ground was complete by October 18. Injury by "Bunker C" was of the chronic type but was probably related to the small root system in the pot culture. Kudzu was rapidly injured by all the lighter oils, but most of the plants recovered by growth of axillary buds from around the crowns. Plants sprayed with stove naphtha were rapidly killed and showed no recovery. Injury by the heavy diesel oil became apparent on September 5 and was complete by September 10. Possibly coverage was more complete than in the case of the two other diesel fractions.

Because the injury by "Varsol" was incomplete and because the volumes applied were not measured in the above experiments, it seemed desirable to repeat the work on pineapples using measured volumes. Table 3 gives the results of this study. The oils were the same except that "Navy Special," a lighter furnace fuel, was substituted for "Bunker C."

From the data of this experiment it seemed that stove naphtha was the only safe oil to use to kill weeds in pineapple fields. Although "Navy Special" did not kill the pineapples, it also was nontoxic to weeds and hence useless. Figure 2 shows the plant that received 5 ml. of "Varsol" in this test. The young leaves in the center were softened at the base, so that they pulled out very easily, and their basal meristem cells were dead. The apical meristem was not killed, however, and a new bud formed and developed, shown in the center of the plant on the left. The plant on the right is a control with the front leaves depressed to show the normal structure of the central portion.

In a test started on October 27, four oils were used, the standard diesel fuel, a no. 2 diesel fuel from Standard Oil of California, "Stanship Diesel"—a heavy marine diesel fuel used to refuel transatlantic diesel freighters—and "Standard Weedkiller no. 2"—a cracked aromatic bottom from California crude sold as a special weed oil. These four oils were tested on pineapples and ten other species. All four were fatal to pineapples.

After these tests a field trial was made using stove naphtha on mature plants of bearing size. For comparative purposes a fortified oil-emulsion spray (4) and a dinitro selective were also used. The stove naphtha was used in sufficient quantity to cover all weeds and pineapple plants. Ten days after application the stove naphtha spray had destroyed the centers of the pineapple plants just as kerosene and diesel oil had done in the greenhouse. The selective and general contact sprays caused a localized injury of a type that had occurred on plants tested with these sprays in the greenhouse and illustrated in figure 3. This injury, although it retarded growth, had no permanent effect. It seems that this type of spray could be safely used in pineapple plantings if care were taken to avoid direct application to the crowns of plants. The tests on oils would seem to indicate that these have no place for weed control in pineapples.

Discussion

The labor involved in weeding constitutes one of the major costs in the production of pineapples. Oil sprays that

kill all weeds, including grasses, also injure pineapples and so cannot be used. Dinitro selectives do not kill grasses, and they cause contact injury to pineapple leaves; there seems to be no place for them. Fortified oil-emulsion sprays will kill all weeds. If care is taken, by keeping the nozzles low and by shielding the pineapple plants, to avoid contact with the young pineapple leaves, weeds can be controlled without injury to pineapples. Preliminary tests prove this to be true. Since such oil sprays are economical, safe, and effective, it seems that their use may effect real savings in weeding costs of this crop.

One further chemical method is under trial, the use of a temporary soil sterilant to kill weed seedlings in the topsoil (7) much as the paper mulch prevents their growth. Though preliminary tests indicate this method to be effective, the ultimate effect of the chemicals in the soil is not known, and the possible variations in leaching following flash rains may result in injury to the crop or may negate the results on the weeds. Much more testing is required before this type of soil treatment can be recommended for general use.

Very little is known concerning the effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on pineapples. VAN OVERBEEK's (9) demonstration of its ability to induce flowering has caused Puerto Rican growers to avoid its use. Greenhouse tests with 2,4-D on young plants of the type illustrated in figures 1, 2, and 3 have not resulted in flowering in Red Spanish plants within 4 months. Though 2,4-D will not kill grasses in pineapple fields, it would prove very convenient to use on other weeds. It seems that comprehensive tests with young plants should be conducted to see whether 2,4-D has any place in weed control in pineapple fields. A pre-planting spray using a fortified oil emulsion to kill all weed growth might prove valuable as a weed-control method. Such a spray used as a pre-emergence treatment in sugar beets has proved effective in California (8).

Summary

1. Hand-weeding is a major item of cost in pineapple production in Puerto Rico.

2. Tests of eight commercial oil fractions on pineapple and weed plants in the greenhouse proved that only the light fraction stove naphtha could be used without injury to pineapple. The use of heavier oils resulted in death and decay of the meristematic tissues of the growing point.

3. Under field conditions even stove naphtha injured mature pineapple plants. A dinitro selective injured pineapple leaves without killing grasses.

4. A fortified oil-emulsion spray may be used, provided the pineapple plants are shielded from direct application.

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LITERATURE CITED

1. COULTER, J. W. La Piña. Union Panamericana Agricola Nos. **134, 135, 136**:1–67. Washington, D.C. 1940.

2. CRAFTS, A. S. Selectivity of herbicides. Plant Physiol. **21**:345–361. 1946.

3. ———. Oil sprays for weeding carrots and related crops. Calif. Agric. Extension Service Circ. 136. 1947.

4. ———. General contact weed killers. Calif. Agric. Extension Service Circ. 137. 1947.

5. CRAFTS A. S., and REIBER, H. G. Herbicidal properties of oils. Hilgardia **18**:77–156. 1948.

6. JOHNSON, M. O. The Pineapple. Paradise of the Pacific Press, Honolulu, T.H. 1935.

7. MONSANTO PROGRESS REPORT. Weed Control with Monsanto Santobrite. Monsanto Chemical Co., St. Louis, Mo. 1948.
8. ROBBINS, W. W., and BAINER, ROY. Preemergence spray for weed control in sugar beets seeded in undisturbed soil successful. Calif. Agric. 1 (No. 11). 1947.
9. VAN OVERBEEK, J. Control of flower formation and fruit size in the pineapple. BOT. GAZ. 108: 64–73. 1946.

SOME EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON STARCH DIGESTION AND REDUCING ACTIVITY IN BEAN TISSUE CULTURES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 603

HAROLD J. F. GALL

The action of several growth-regulating substances in accelerating digestion of starch in leaves, stems, and roots of bean and other plants has been reported (1, 12, 19). The present note records the use of 2,4-dichlorophenoxyacetic acid (2,4-D) in culturing stem tissue of bean on an agar medium containing starch as the sole source of carbohydrates, and observations on reducing activity in these tissues. Procedures followed were partly indicated by earlier work by GROSSE (4) with similar material. Experiments with thin sections made from stems of bean plants previously treated with indoleacetic acid have been described by BEAL (2) and with sections treated with 2,4-D *in vitro* by SMITH (17).

The nutrient solution used was, omitting sucrose, that of RIKER and GUTSCHE (13), composed as follows: water, redistilled in glass, 1000 ml.; Na_2SO_4, 100 mg.; KCl, 130 mg.; $NaH_2PO_4 \cdot H_2O$, 132 mg.; $MnSO_4 \cdot 4H_2O$, 4.5 mg.; $ZnSO_4 \cdot 7H_2O$, 3 mg.; H_3BO_3, 3 mg.; KI, 0.38 mg.; $MgSO_4 \cdot 7H_2O$, 720 mg.; $Ca(NO_3)_2 \cdot 4H_2O$, 800 mg.; KNO_3, 160 mg.; thiamine hydrochloride, 0.1 mg.; pyridoxine, 0.8 mg.; ferric tartrate, 5 mg. In preparing the medium, 10 gm. of Baker & Adamson's soluble starch and 5 gm. of Difco Bacto-Agar were added to a liter of nutrient solution and the whole autoclaved for 20 minutes. After thorough stirring, 25 ml. of the hot solution were added by burette to sterile 90-mm. Petri dishes. One series received in addition 10 mg. of 2,4-D per liter, added to the solution before autoclaving.

Young plants of Red Kidney bean, grown in soil in the greenhouse until the first trifoliate leaves had just begun to expand, provided the culture material. Adequate sterilization, without apparent injury to the tissues, was achieved most satisfactorily by use of bromine. After the leaves had been removed from the plants, the stems were cut below the cotyledonary node and placed immediately in 1% bromine water containing about 0.1% Aerosol O.T. as a wetting agent. They were removed after 1 minute and allowed to drain on sterile filter paper in closed Petri dishes for 15–20 minutes. Sections approximately 1.5 mm. in thickness were then cut with a sterile scalpel from the internode and placed in the culture dishes on the agar surface, with the basal end downward. The fresh weight of these sections was 9 mg. ± 2.

The air of the laboratory room in which cultures were prepared was sterilized by means of triethylene glycol (14) vaporized in a Stokes vaporizer (5)

kindly supplied by Dr. O. H. ROBERTSON. Satisfactory results were regularly obtained by this method when supplemented by dust-suppressive measures. For general use in tissue-culture work the possible growth-affecting activity of the glycol vapor should be considered. In this experiment any such effects could be disregarded.

The culture dishes were kept in the dark, except for brief intervals of examination, in a thermostatically controlled chamber at 26° C. ± 1. After 2 days under these conditions sections growing on plus–2,4-D medium showed marked proliferation on the upper surface, with the cortical region expanding. Sections on minus–2,4-D medium showed a slight superficial proliferation, with little or no cortical expansion, and a general browning of the cut surface. After 4 days the plus sections were still greenish in color, with the proliferated areas nearly colorless. The epidermis had split into five to eight strongly reflexed segments. Four sections examined weighed 29.8, 28.6, 30.0, and 34.2 mg., respectively. After 4 days the minus–2,4-D sections were brown, had the epidermis intact, and showed no additional proliferation. Four of these sections weighed 12.2, 8.4, 12.8, and 10.0 mg., respectively.

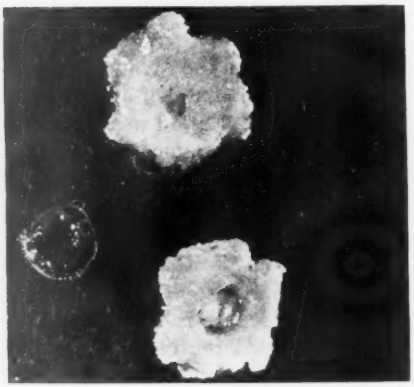

FIG. 1.—Sections of bean stem cultured 15 days on nutrient agar containing 1% starch and, *left*, no 2,4-D; *upper*, 10 mg./l. 2,4-D (top view); *lower*, 10 mg./l. 2,4-D (bottom view). ×3.

After 15 days the plus–2,4-D cultures had become irregularly rounded masses of unorganized whitish tissue (fig. 1). No proliferation had taken place at that surface of the section in contact with the agar. Little or no outward change was apparent in the minus–2,4-D sections. Fresh weights of four sections of each group were 56.4, 56.8, 42.6, and 62.4 mg. and 12.2, 14.2, 14.8, and 14.4 mg., respectively. At this time the culture dishes were stained with iodine to show the pattern of starch degradation in the medium. The dishes were flooded with dilute Lugol's solution, allowed to stand for 20 minutes, then drained and washed with cold water. Figure 2 illustrates three stained cultures, taken at random before staining from each of the two series, from initial numbers of thirty-eight and thirty-nine dishes for the plus–2,4-D and the minus–2,4-D series, respectively. The affected areas of the former group are not only of greater extent but also more sharply defined and the colorless regions clearer than those of the latter group. The zone of transition from the unaffected to the completely colorless areas exhibited the sequence of colors with iodine, from blue through violet, rose, and orange, characteristic of a series of reducing dextrins of decreasing complexity.

The type of response induced in bean stem tissue by 10 mg. of 2,4-D per liter outwardly resembles that described (15) for normal sunflower stem tissue in which a like concentration of naphthalene acetic acid caused a disorganized [IC-

liferation of cambial origin. Preliminary histological examination indicated the proliferation in the bean stem sections to be largely of the endodermis, cambium, and phloem, in line with observed responses of bean plants to 2,4-D (**18**). Pathological sunflower tissue was strongly inhibited by 2,4-D even at dilutions distinct and less extensive pattern of digestion. The condition of the starch grains after the particular solubilizing process used is apparently a factor in the amount and kind of degradation effected.

Starch degradation in the minus–2,4-D medium may be considered the result of the action of an enzyme or en-

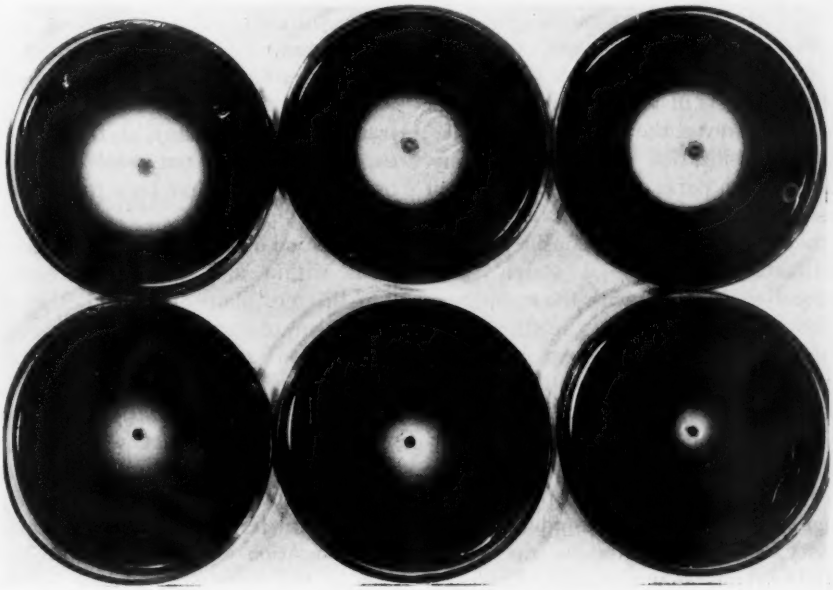

FIG. 2.—Patterns of starch degradation in cultures of bean stem tissue. *Upper row*, medium with 10 mg./l. 2,4-D; *lower row*, medium without 2,4-D.

as high as 1×10^{-13} gm. per liter, and tobacco tissue was inhibited at dilutions of 1×10^{-5} to 1×10^{-7} (**7**). In contrast to the response described here for bean stem sections, SMITH (**17**) observed little stimulated meristematic activity in thin slices (400–800 μ) of bean stem incubated 48 hours with 2,4-D.

It should be noted that the Baker & Adamson's soluble starch used here results in a clear solution when heated. Other soluble starches which result in a milky solution when heated gave a less zymes entering the agar from the wounded surfaces of the fresh stem sections. The positive effect reported here of 2,4-D in enhancing the degradation of starch *in vitro* may be the result of direct action upon the amylase or phosphorylase systems or both, causing an increase in production or activity of the enzymes. Mechanical disruption of the tissues in the early stages of proliferation or alteration of permeability of cell membranes may be factors involved, as resulting in greater leakage of enzyme from the stem

sections cultured with, than from those cultured without, 2,4-D. The parallel with disappearance of starch from cells of living intact plants treated with growth substances is at least suggestive that a metabolic rather than merely mechanical disturbance may be operative and that the two occurrences may be manifestations of a single underlying effect. Correlation of starch disappearance with new cell growth also obtains both in treated intact plants (**1, 19**) and in the treated stem sections of this experiment. In the latter, however, the picture is simplified, since mobilization of reserve carbohydrates from parts of the plant and their utilization in meristematic activity in other parts need not be considered. The intimate association of starch phosphorolysis in plants with the respiratory sequence (**6, 8**) suggests relations that might profitably be explored with reference to the mode of action of 2,4-D and other growth substances.

In this connection it may be significant that the tissues involved in the growth response are those possessing a high level of oxidation-reduction activity, as demonstrated by the use of triphenyl tetrazolium chloride. This compound, colorless in aqueous solution, may be enzymatically reduced in living cells to the insoluble red triphenyl formazan (**3, 11**). Sections 1.5 mm. in thickness cut from the first internode of young bean plants and placed in 0.5% triphenyl tetrazolium chloride solution were deeply stained red within 2 hours. Microscopic examination showed the red formazan to be sharply localized in the endodermis, the phloem (particularly in the parenchyma cells surrounding the tannin ducts), the cambium, where this could be distinguished, and the xylem parenchyma surrounding the vessels. This pattern of enzyme activity is thus strikingly similar to the pattern of meristematic activity shown by KRAUS *et al.* (**10**) in sections made from bean plants 30 hours after treatment with indoleacetic acid. Tissues of older plants, in which the first internode had ceased to elongate, gave a much less intense reaction with tetrazolium chloride. When sections from these plants were cultured for 14 hours on plus- and minus-2,4-D media containing 1% sucrose, an intensification of reducing activity, especially in the endodermis, was apparent in many of the sections exposed to 2,4-D, but results in these preliminary tests have been too variable to be conclusive.

Under oil immersion the red-stained areas within individual cells resolved into discrete filamentous bodies in the cytoplasm, of varying form and size, interpreted to be mitochondria. Nuclei and nucleoli showed no evidence of staining. The intracellular localization of intense reducing activity to the mitochondria, as these findings suggest, is of particular interest in the light of recent biochemical work on rat liver (**9, 16**) demonstrating the association with isolated mitochondria of the fatty acid oxidase system and of certain enzyme systems of the Krebs tricarboxylic acid cycle. According to MATTSON *et al.* (**11**), reduction of triphenyl tetrazolium chloride in living tissues is probably effected by dehydrogenase systems requiring coenzymes I or II. It would be useful to study mitochondria isolated from plant material with regard to dehydrogenase and other enzyme activity and the action of growth substances.

Summary

Sections of bean stem cultured on nutrient agar containing 1% starch and 10 mg. per liter of 2,4-D showed extensive

proliferation after 15 days. Areas of starch degradation in the medium containing 2,4-D were clearer and more extensive than in medium without 2,4-D. Reducing activity of the endodermis, phloem, cambium, and xylem parenchyma was demonstrated by the use of triphenyl tetrazolium chloride.

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LITERATURE CITED

1. BAUSOR, S. C. Effects of growth substances on reserve starch. BOT. GAZ. **104**:115–121. 1942.
2. BEAL, J. M. Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. BOT. GAZ. **102**:366–372. 1940.
3. DUFRENOY, J., and PRATT, R. Histophysiological localization of the site of reducing activity in stalks of sugar cane. Amer. Jour. Bot. **35**:333–334. 1948.
4. GROSSE, SHIRLEY H. Effects of 2,4-dichlorophenoxyacetic acid in combination with organic metabolites on bean stem cultures. Thesis. Univ. Chicago. 1947.
5. HARRIS, T. N., and STOKES, J., JR. A simple vaporizing device for the attainment of bactericidal concentrations of glycol vapors in air. Science **104**:423–425. 1946.
6. HASSID, W. Z. The molecular constitution of starch and the mechanism of its formation. Quart. Rev. Biol. **18**:311–330. 1943.
7. HILDEBRANDT, A. C., and RIKER, A. J. Influence of some growth-regulating substances on sunflower and tobacco tissue *in vitro*. Amer. Jour. Bot. **34**:421–427. 1947.
8. JAMES, W. O. The respiration of plants. Ann. Rev. Biochem. **15**:417–434. 1946.
9. KENNEDY, E. P., and LEHNINGER, A. L. Intracellular structures and the fatty acid oxidase system of rat liver. Jour. Biol. Chem. **172**:847–848. 1948.
10. KRAUS, E. J.; BROWN, N. A.; and HAMNER, K. C. Histological reactions of bean plants to indoleacetic acid. BOT. GAZ. **98**:370–420. 1936.
11. MATTSON, A. M.; JENSEN, C. O.; and DUTCHER, R. A. Triphenyltetrazolium chloride as a dye for vital tissues. Science **106**:294–295. 1947.
12. MITCHELL, J. W., and WHITEHEAD, MURIEL R. Starch hydrolysis in bean leaves as affected by application of growth-regulating substances BOT. GAZ. **102**:393–399. 1940.
13. RIKER, A. J., and GUTSCHE, ALICE E. The growth of sunflower tissue *in vitro* on synthetic media with various organic and inorganic sources of nitrogen. Amer. Jour. Bot. **35**:227–238. 1948.
14. ROBERTSON, O. H. New methods for the control of air-borne infection with especial reference to the use of triethylene glycol vapor. Wis. Med. Jour. **46**:311–317. 1947.
15. DE ROPP, R. S. The response of normal plant tissues and of crown-gall tumor tissues to synthetic growth hormones. Amer. Jour. Bot. **34**: 53–62. 1947.
16. SCHNEIDER, W. C. Intracellular distribution of enzymes. III. The oxidation of octanoic acid by rat liver fractions. Jour. Biol. Chem. **176**:259–266. 1948.
17. SMITH, F. G. The effect of 2,4-dichlorophenoxyacetic acid on the respiratory metabolism of bean stem tissue. Plant Physiol. **23**:70–83. 1948.
18. SWANSON, C. P. Histological responses of the kidney bean to aqueous sprays of 2,4-dichlorophenoxyacetic acid. BOT. GAZ. **107**:522–531. 1946.
19. TUKEY, H. B.; HAMNER, C. L.; and IMHOFE, BARBARA. Histological changes in bindweed and sow thistle following applications of 2,4-dichlorophenoxyacetic acid in herbicidal concentrations. BOT. GAZ. **107**:62–73. 1945.

CONTROL OF MILDEW ON BEAN PLANTS BY MEANS OF AN ANTIBIOTIC[1]

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By way of participating in the screening program of the National Research Council, Chemical-Biological Coordination Center, to secure information about newly developed chemical compounds with regard to their possible growth effects on higher plants, an antibiotic substance was received among other materials for testing. In the routine test this substance, actidione, proved to be toxic in concentrations higher than 100 p.p.m. to young bean plants and oat seedlings.

Actidione is a product of the Upjohn Company, Kalamazoo, Michigan. It is isolated from the beers in which streptomycin-producing strains of *Streptomyces griseus* are cultivated. This antibiotic has been credited (**2, 3**) with antifungal activity, inhibiting the growth of yeast and some pathogens of man.

Incidentally, at the time when actidione was tested for its growth-regulating properties, older bean plants (Red Kidney) in the greenhouse became severely infected with powdery mildew. The generally accepted treatments with sulfur failed to control the pest. Then it occurred to the authors that actidione might also have activity against fungi that cause plant diseases. When an aqueous solution of actidione at 10 p.p.m. was sprayed on the plants affected by mildew, the grayish-white patches on the upper surface of the leaves disappeared completely within 48 hours, indicating that the mycelium and the conidia of the fungus *Erysiphe polygoni* D.C. had been destroyed.

Subsequent to this preliminary result, experiments were conducted with several hundred bean plants for the purpose of finding the lowest concentration at which actidione still would be effective against mildew, and also to determine what substance could be used advantageously as a carrier and spreader with the material. Concentrations of 10, $7\frac{1}{2}$, 5, $2\frac{1}{2}$, and 1 p.p.m. were tried. Carbowax, Triton X-155, and methylcellulose were tested as carriers. At the present time the most satisfactory formulation seems to be an aqueous dispersion of 0.25% methycellulose (1500 cp. viscosity type) to which is added 5 p.p.m. of actidione. Methycellulose used alone at concentrations between 0.25 and 1% neither prevented nor controlled the infection. No difference in effect was noticeable when the spray solution was made up with either distilled or tap water.

Since lesions of the tissues remain after the leaves once have been attacked by mildew and since these restrict the growth of the plant even after the parasite has been destroyed, it seemed advisable to use actidione prophylactically. If the first spray application was made to the primary leaves of bean plants when 10 or 12 days old and if treatments were then continued in intervals of 6–10 days corresponding to the production rate of the trifoliate leaves, these plants were protected during their lifetime against mildew, even when closely surrounded by heavily infected control plants. Actidione in concentrations of 10 p.p.m. or lower has no apparent effect on the growth of

[1] Journal Article no. 975 (N.S.) of the Michigan Agricultural Experiment Station.

the bean plant itself at its various stages of development.

The fungal spectrum for other organisms which cause plant diseases is being investigated. It seems possible that actidione may be active against other fungal plant pathogens that damage crops and reduce yields. Fungicidal aerosols have not yet been used successfully in disinfecting practices. The use of an antibiotic as highly active in such minute amounts as actidione, however, has possibilities for application in form of an aerosol that may greatly facilitate control in greenhouses, food storages, etc. Preliminary tests in animals (1) would indicate the nontoxicity of actidione in the concentrations here reported.

The important fact is that actidione, which is an antibiotic substance derived from a fungus, exhibits a growth-inhibiting effect on another plant parasite and in amounts as small as from 1 to 5 p.p.m. This suggests the possibility of utilizing antibiotic substances in large-scale industrial and field operations for the control of fungus diseases of plants.

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LITERATURE CITED

1. FORD, J. H., and LEACH, B. E. Actidione, an antibiotic from *Streptomyces griseus*. Jour. Amer. Chem. Soc. **70**:1223–1226. 1948.

2. LEACH, B. E.; FORD, J. H.; and WHIFFEN, ALMA J. Actidione, an antibiotic from *Streptomyces griseus*. Jour. Amer. Chem. Soc. **69**:474–475. 1947.

3. WHIFFEN, ALMA J.; BOHONES, N.; and EMERSON, R. L. The production of an antifungal antibiotic by *Streptomyces griseus*. Jour. Bact. **52**:610–611. 1947.

SIZE-FREQUENCY CHARACTERISTICS OF PINUS ECHINATA POLLEN

STANLEY A. CAIN AND LOUISE G. CAIN

Introduction

Students of fossil pollen have realized the desirability of exact identification of the species represented in fossil and subfossil floras because of the occasional great differences in ecological and geographic significance of the different species of a single genus. They have in the main found it necessary, however, to content themselves with generic determinations because of the close structural similarity of the pollen grains of the species, for example, of spruce, fir, pine, oak, and birch.

The senior author became interested in the problem of specific determination of pollen grains while investigating the flora of some buried soils in the Piedmont of South Carolina (4) under the auspices of the Physiographic and Climatic Research Division of the Soil Conservation Service. These buried soils contained a considerable element of coniferous pollen, including more spruce and fir than seemed reasonable to have resulted from long-distance dispersal, and a representation of a variety of pine pollen. European investigators (1, 19, 24, 25) had employed size-frequency or other biometric data in an effort to distinguish species of pine and birch, so an effort was made along these lines in connection with the

problem of buried soils (3). Interest in the size-frequency technique was stimulated by the probable identification of fossil pollen of *Pinus banksiana* from South Carolina, a species today having the most northern range of any American pine and having its southern limits several hundred miles from the fossil site.

As a novice in the field of pollen analysis, the senior author was impressed both by the great possibilities of such studies for the reconstruction of vegetational and climatic history and by the numerous difficulties and pitfalls attendant on such reconstructions (2, 5, 7). This interest resulted in a study of the effect of different types of chemical treatment on the size characteristics of pollen. For this study pollen of *Abies fraseri* was used (6), and it was shown that the different techniques have significant effects on grain size. Attacking another basic problem, a student (12) undertook a study of over- and under-representation of various pollen types in the pollen rain under a section of virgin spruce-fir forest in the Great Smoky Mountains. Similar studies are much needed elsewhere under different conditions and with different species.

The original survey of the size characteristics of the pollen of twelve species of eastern American pines (3) was based on a single sample of each species. In many cases the size-frequency curves were so similar for certain species that there seemed little hope for the identification of species by such methods if the character were subject to much variation. Consequently, a request was made to field workers of the Soil Conservation Service for fresh collections of pine pollen so that samples of a wide geographic representation of at least some of the species could be examined. Since the acquisition of these materials nearly thirty thousand grains have been measured, mostly by LOUISE G. CAIN: A study of *Pinus palustris* has already been published (9), and the material for modern grains of *P. banksiana*, *P. resinosa*, and *P. strobus* has been used in tracing the probable postglacial successional development of these pines in the vicinity of Sodon Lake, Oakland County, Michigan (10). Here, also, a size-frequency study of modern and fossil grains of spruce has led to the suggestion that the eastern *Picea rubra* was probably present in the western Great Lakes area at an earlier time (8, 11). In America the size-frequency technique has also been applied to grass pollen (17, 21, 23) in a largely unsuccessful effort to distinguish aquatic grasses from possible indicators of the subboreal xerothermic period. DEEVEY (13) thought the size-frequency technique worthless in his studies on Connecticut sediments, but HANSEN (18) has made extensive and apparently successful use of the size-frequency characteristics of pollen grains in his monographic study of forest history in the Pacific Northwest.

The present study of *Pinus echinata* Mill. is based on twenty-seven samples which were collected from Texas to New Jersey, covering much of the area of the species (fig. 1). Material from each collection was prepared for examination by the ERDTMAN acetolysis method (2, 15, 16) since that method is also used by the authors for the preparation of fossil pollen. For each collection only one hundred fifty grains were measured, as other studies (9) had shown that number to be adequate for statistical comparisons. The data are presented in table 1 and figure 2.

Results

In table 1 the grains are seen to have an extreme variation from about 42 to 70 μ. The data are presented as percent-

age frequencies in nineteen size classes, each of which represents one scale unit on the ocular micrometer (at 440 ×), or 1.56 μ. All grain measurements are comparable, since they represent the greatest intra-exine dimension of the tube cell as seen squarely in dorsal, ventral, or lateral view. The table also shows the mean grain size ($\overline{X}$) and the standard deviation (δ) for each collection. The data for the collections are arranged in an array according to the value of the mean. Figure 2 is a graphic representation (arranged in the same sequence as table 1) of certain biometrical data concerning the different collections. For each unit of the diagram the horizontal line represents the extreme size range of the grains of the collection. The vertical line marks the position of the mean ($\overline{X}$). The open rectangle sets off $\overline{X} \pm$ one standard deviation (δ), and the black bar marks $\overline{X} \pm 2$ standard errors ($\delta/\sqrt{N}$). This graphic technique, from DICE and LERAAS (14) and HUBBS and PERLMUTTER (20), is very useful, for it shows at a glance whether certain sets of measurements have statistically significant differences, since the odds against the occurrence of a deviation as great or greater than two standard errors are about 21 to 1, and three standard errors are about 370 to 1 (22).

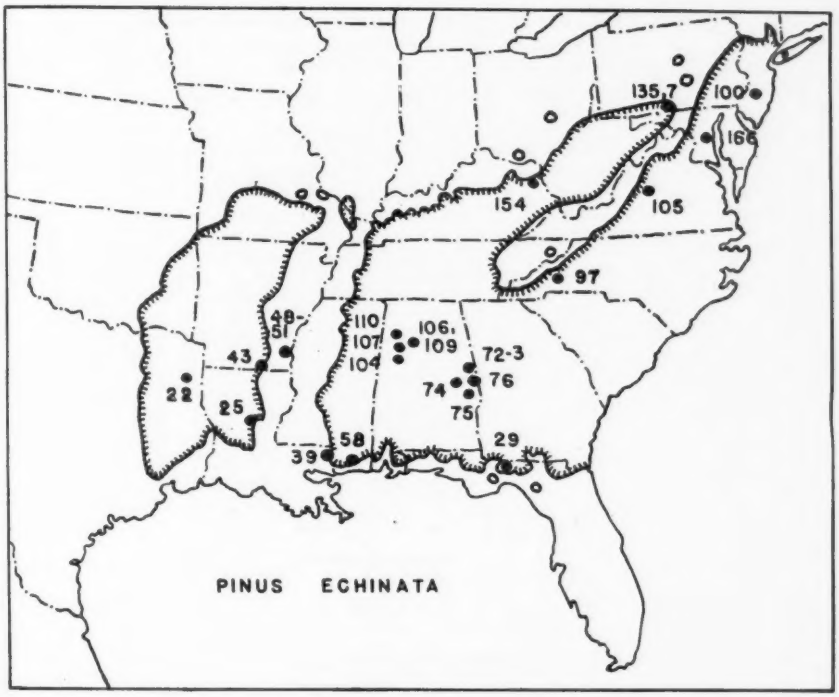

FIG. 1.—Range of *Pinus echinata* and points of collection of pollen

This study of *Pinus echinata* shows what is coming to be expected of size characteristics of morphological features in general, that is, a considerable intraspecific variability. Some collections from stations considerably removed from one

another may be very similar (coll. 48, Arkansas; coll. 73, Alabama). Other materials from one locality (Alabama collections 109 and 110) may be significantly different.

A feature of interest that we have not encountered in our previous pollen studies is some evidence for a geographic cline of pollen size in *Pinus echinata*. Although the data of table 1 and figure 2, arranged in an array by $\bar{X}$ sizes, show only a gradual change and no "saltation," it is possible to recognize certain geographic tendencies. The center for small grains seems to be in the central Gulf States. In fact, if we treat as one group the twenty collections from the southern half of the area of the species and as another group the seven collections from the northeastern part of the range, we find that the means of the latter group are all larger than any of the former group. This division is the only consistent one that can be made both on the map (fig. 1) and in the array (fig. 2 and table 1). There is some suggestion that grain size is also larger on the western periphery of the area of the species

TABLE 1

SIZE-FREQUENCY CHARACTERISTICS OF POLLEN GRAINS OF *Pinus echinata*. DATA BASED ON 150 GRAINS IN EACH COLLECTION

Collection no.	State	Size in microns		Percentage occurrence of grains																				
		$\bar{X}$	δ	42.1*	43.7	45.2	46.8	48.4	49.9	51.5	53.0	54.6	56.2	57.7	59.3	60.8	62.4	64.0	65.5	67.1	68.6	70.2
				Smaller grains of southern area of species																		
104	Ala.	48.71	± 2.78	1.3	5.3	11.3	14.0	26.0	17.3	13.3	9.3	1.3	0.7									
76	Ala.	50.22	± 2.59	0.7		4.0	6.6	22.7	30.0	15.3	10.0	8.7	0.7	1.3								
39	La.	51.11	± 2.22			1.3	5.3	8.6	27.3	33.3	10.0	12.0	2.0									
109	Ala.	51.32	± 2.59			2.0	8.0	8.7	20.7	25.3	19.3	12.0	2.7	0.7	0.7							
49	Ark.	51.77	± 2.59			1.3	4.0	10.6	18.7	22.7	18.0	16.7	7.3	0.7								
107	Ala.	51.99	± 2.81			1.3	4.6	8.7	20.0	18.7	20.0	15.3	7.3	3.3	0.7							
48	Ark.	52.16	± 2.29				2.7	5.3	20.0	24.7	20.7	19.3	6.6	0.7								
73	Ala.	52.16	± 2.45				3.3	9.3	16.0	21.3	21.3	20.0	8.0	0.7								
50	Ark.	52.24	± 2.37		0.7		2.0	7.3	13.3	27.3	22.7	19.3	5.3	2.0								
106	Ala.	52.56	± 2.67				2.0	6.6	18.7	19.3	21.3	16.7	10.0	4.0	1.3							
29	Fla.	52.67	± 2.40				2.0	4.0	11.3	24.7	24.0	20.7	6.6	3.3	1.3							
74	Ala.	52.80	± 2.26				0.7	4.0	12.0	26.0	24.0	20.7	10.6	0.7	1.3							
58	Miss.	52.82	± 1.98					2.7	16.0	18.7	30.7	22.7	6.6	2.0	0.7							
25	La.	52.84	± 2.17				1.3	2.0	12.6	22.0	32.7	16.7	9.3	3.3								
43	Ark.	53.31	± 2.65				1.3	1.3	16.0	16.7	22.0	23.3	10.6	6.0	1.3	0.7	0.7					
72	Ala.	54.09	± 2.24					0.7	6.0	14.0	24.0	28.7	15.3	10.0	0.7	0.7						
75	Ala.	54.42	± 2.45					2.0	3.3	14.0	18.0	32.0	16.0	8.6	4.6	1.3						
110	Ala.	54.43	± 2.62				0.7		5.3	15.3	20.0	26.7	16.7	8.0	4.6	2.0	0.7					
51	Ark.	54.54	± 2.53				0.7	0.7	6.6	10.0	16.7	28.7	20.7	11.3	3.3	1.3						
22	Tex.	54.62	± 2.54					2.0	3.3	12.0	20.0	27.3	15.3	12.6	6.6	0.7						
				Larger grains of northeastern area of species																		
166	Md.	55.05	± 2.78					2.0	2.7	11.3	16.7	24.7	16.7	15.3	7.3	2.0	1.3					
154	Ky.	55.83	± 2.99				0.7	2.0	2.0	6.7	12.0	22.0	14.7	20.0	14.7	4.0	1.3					
137	Pa.	56.29	± 2.59					0.7	0.7	4.0	10.0	20.7	26.7	17.3	12.6	4.6	2.7					
135	Pa.	56.84	± 2.53							2.7	7.3	20.0	22.7	22.7	16.0	5.3	2.0	0.7	0.7			
105	Va.	57.08	± 3.90						3.3	5.3	7.3	15.3	16.7	24.7	10.6	4.6	6.0	3.3	1.3	0.7	0.7	
100	N.J.	57.56	± 2.76					0.7	0.7	2.7	5.3	11.3	16.7	23.3	20.7	14.0	4.0	0.7				
97	N.C.	60.03	± 4.52					0.7		1.3	3.3	3.3	12.0	16.0	20.0	12.0	9.3	8.6	7.3	0.7	4.0	1.3
Southern group, all grains		52.53	± 2.95	0.1	0.3	1.1	2.9	6.8	14.7	19.7	20.2	19.4	8.9	3.9	1.4	0.3	0.1					
Northern group, all grains		56.96	± 3.38				0.1	0.9	1.3	4.9	8.9	16.8	18.0	19.9	14.6	6.6	3.8	1.9	1.3	0.2	0.7	0.2

* Size classes in microns.

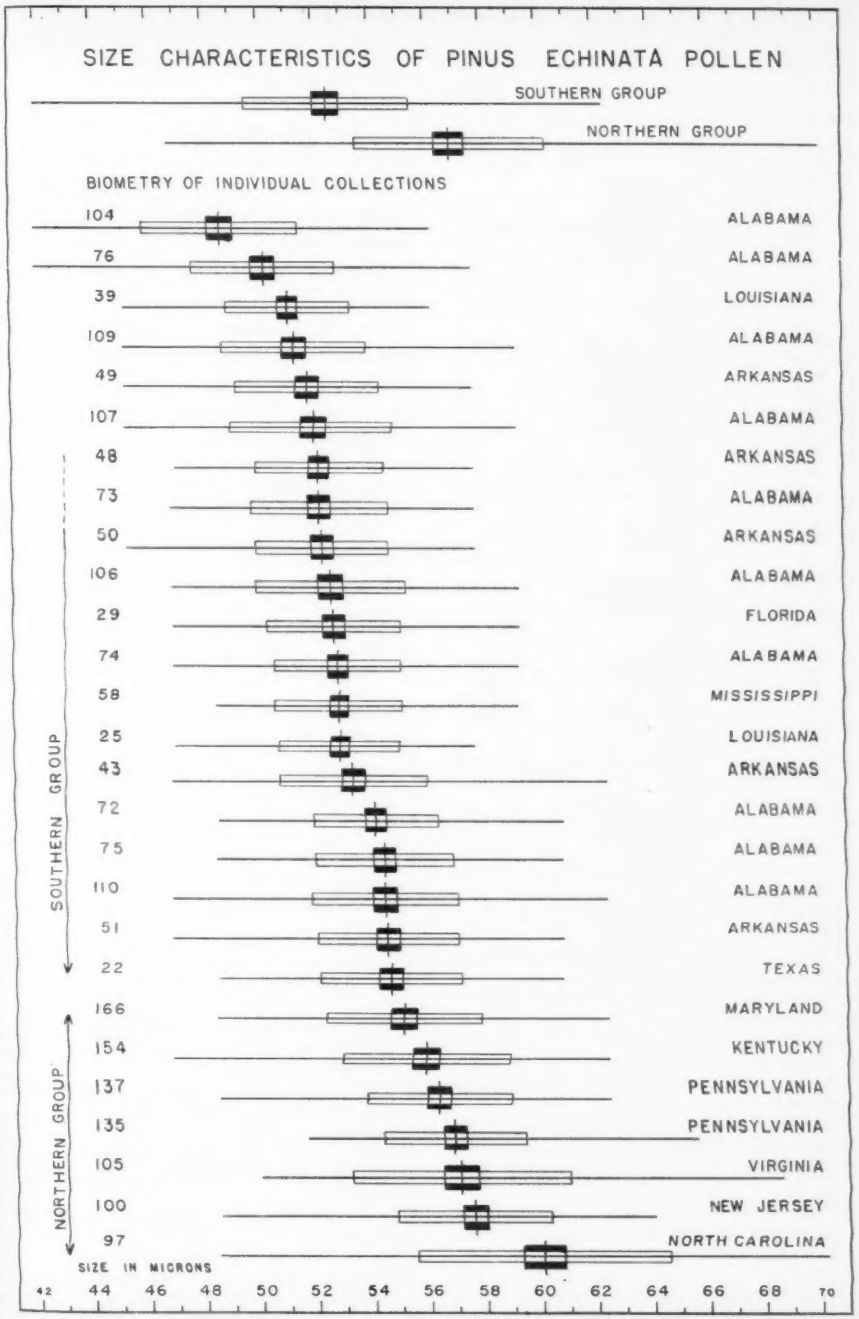

FIG. 2.—See explanation in text

(Texas), as it is on the eastern (North Carolina), but the collections are inadequate to demonstrate this conclusively. The size data are summarized by states in table 2.

TABLE 2

MEAN POLLEN GRAIN SIZES (μ) OF *Pinus echinata* ARRANGED BY STATES

Southern area of species		Northeastern area of species	
Louisiana	51.97	Maryland	55.05
Alabama	52.27	Kentucky	55.83
Florida	52.67	Pennsylvania	56.57
Arkansas	52.80	Virginia	57.08
Mississippi	52.82	New Jersey	57.56
Texas	54.62	North Carolina	60.03
Southern group	52.53	Northern group	56.96

Conclusions

Different collections of pollen grains of *Pinus echinata* may be similar as to size characteristics from stations a few hundred miles apart or be significantly different from stations only a few miles apart.

There is evidence in the data for the existence of a geographic cline of pollen-grain size in *Pinus echinata*, with the grains of the northeastern half of the area of the species having consistently larger mean values than those of the southern half of the area of the species.

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LITERATURE CITED

1. BERTSCH, K. Paläobotanische Monographie des Federseerieds. Bibliotheca Bot. **26** (103): 1–127. 1931.
2. CAIN, S. A. Pollen analysis as a paleo-ecological research method. Bot. Rev. **5**:627–654. 1939.
3. ———. The identification of species in fossil pollen of *Pinus* by size-frequency determinations. Amer. Jour. Bot. **27**:301–308. 1940.
4. ———. Pollen analysis of some buried soils, Spartanburg County, South Carolina. Bull. Torrey Bot. Club **71**:11–22. 1944.
5. ———. Foundations of Plant Geography, esp. chap. 10. Harper & Bros., New York. 1944.
6. ———. Size-frequency characteristics of *Abies Fraseri* pollen as influenced by different methods of preparation. Amer. Midl. Nat. **31**:232–236. 1944.
7. ———. The place of pollen analysis in paleoecology. Chron. Bot. **9**:106–114. 1945.
8. ———. Palynological studies at Sodon Lake. I. Size-frequency study of fossil spruce pollen. Science **108**:115–117. 1948.
9. CAIN, S. A., and CAIN, LOUISE G. Size-frequency studies of *Pinus palustris* pollen. Ecology **25**: 229–232. 1944.
10. ———, and ———. Palynological studies at Sodon Lake. II. Size-frequency studies of pine pollen, fossil and modern. Amer. Jour. Bot. **35**:583–591. 1948.
11. CAIN, S. A., and SLATER, J. V. Palynological studies at Sodon Lake. III. The sequence of pollen spectra, profile I. Ecology. **29**:492–500. 1948.
12. CARROLL, GLADYS. The use of bryophytic polsters and mats in the study of recent pollen deposition. Amer. Jour. Bot. **30**:361–366. 1943.
13. DEEVEY, E. S. Studies on Connecticut lake sediments. I. A postglacial climatic chronology for southern New England. Amer. Jour. Sci. **237**: 691–724. 1939.
14. DICE, L. R., and LERAAS, N. J. A graphic method for comparing several sets of measurements. Contr. Lab. Vert. Gen., Univ. Mich., **3**:1–3. 1936.
15. ERDTMAN, G. New methods in pollen analysis. Svensk. Bot Tidsskrift **30**:154–164. 1936.
16. ———. An Introduction to Pollen Analysis. Chron. Bot., Waltham, Mass. 1943.
17. GEISLER, FLORENCE. A study of pollen grains of thirty-two species of grasses. Butler Univ. Bot. Studies **7**:65–73. 1945.
18. HANSEN, H. P. Postglacial forest succession, climate, and chronology in the Pacific Northwest. Trans. Amer. Phil. Soc. (N.S.) **37**:1–130. 1947.
19. HÖRMANN, H. Die pollenanalytische Unterscheidung von *Pinus montana*, *P. silvestris*, und *P. cembra*. Oesterr. Bot. Zeit. **78**:215–228. 1929.
20. HUBBS, C. L. and PERLMUTTER, A. Biometric comparison of several samples, with particular reference to racial investigations. Amer. Nat. **76**:582–592. 1942.
21. KELLER, C. O. A comparative study of three Indiana bogs. Butler Univ. Bot. Studies **6**:65–80. 1943.
22. PEARL, RAYMOND. Medical Biometry and Statistics. W. B. Saunders Co., Philadelphia, 1930.
23. POTZGER, J. E., and THARP, B. C. Pollen profile from a Texas bog. Ecology **28**:274–280. 1947.
24. STARK, P. Ueber die Zugehörigkeit des Kiefer-pollens in den verschiedenen Horizonten der Bodenseemoore. Bot. Ges. **45**:40–47. 1927.
25. ———. Ueber die Diagnose des Birkenpollens in fossilen Fundschichten. Die Naturwiss. **17**: 903–904. 1929.

CURRENT LITERATURE

Bibliography of the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition, Vol. 1. 4th ed. Compiled and published by the CHILEAN NITRATE EDUCATIONAL BUREAU, INC. (120 Broadway, New York 5), 1948. Pp. iii+ 1037.

All previous editions, including the seven supplements of the third, are combined into a single volume of 1976 columns, thereby increasing the usefulness of this well-known work of abstracts. All entries were assembled and reproduced by the offset lithography process. New material completes the bibliography to June 30, 1947. As in previous editions, abstracts are complete with literature citations and are placed under forty-five chemical elements which are arranged alphabetically from aluminum to zinc. Four indexes—author, element, animal nutrition, and botanical—are reasonably complete, with the exception of the very diversified botanical index. All research specialists and alert teachers in plant and animal science will find the present compilation indispensable for successful work and a broad understanding of inorganic nutrition.

In anticipation of a strong interest in the functions of iodine and sodium in determining the nutritive qualities of food and feed crops, the sections pertaining to these elements have been reprinted as a separate publication.—PAUL D. VOTH.

Growth of Plants: Twenty Years' Research at Boyce Thompson Institute. By WILLIAM CROCKER. New York: Reinhold Publishing Corp., 1948. Pp. v+ 459+171 figs.+48 tables. $10.

A critical summary of 20 years of research work on the growth and productivity of plants at Boyce Thompson Institute for Plant Research at Yonkers, New York, is presented by the managing director. No attempt is made to review the results obtained by workers at other institutions except where a statement of these results is necessary to orient the work of the Institute generally to the whole field of plant science.

As stated in the Introduction, the founder, Colonel WILLIAM BOYCE THOMPSON, who provided the endowment, had been impressed with the social value of research. He believed that one of the best investments that could be made for society was properly chosen and directed research and that any principles concerning the nature of life that can be established for plants will help one to understand man in health and disease. He was impatient with the ineffectiveness and unreality of the political approach to national problems and the waste and stupidity of politics. He saw hope only in an order based on economics illuminated and disciplined by science. He planned an institution to deal with plant physiology, plant pathology, and related subjects to help protect the basic needs of the two hundred million people which he envisioned as the future population of the United States.

The plans of the director and his colleagues are also stated in the Introduction. In brief they are: (1) any project or problem tackled should be studied in all its relations, including its meaning in nature, in agriculture, and in industry; (2) it is desirable to organize to attack problems by focusing enough different techniques on the projects to bring evidence on them from many, if not from all, angles; (3) adequate equipment for effective work must be provided even though it may involve very large expenditures; (4) competent technicians and helpers for the investigators must be provided; (5) prompt publication is desirable; and (6) the main fields of research for the Institute should be plant physiology, pathology, and biochemistry.

The larger projects with their many scientific and practical ramifications and the solution of most of the problems are stated in the first eleven chapters. The chapter headings are: (1) "Early problems (yellows and virus diseases of plants, duck food problem, and study of aquatic plants)"; (2) "Life span of seeds"; (3) "Dormancy in seeds"; (4) "Physiological effects of ethylene and other unsaturated carbon-containing gases"; (5) "Effect of certain lethal gases upon plants and animals"; (6) "Plant hormones" (by P. W. ZIMMERMAN); (7) "Dormancy in buds"; (8) "Plant cell membranes" (by WANDA K. FARR); (9) "Plants grown under controlled environmental conditions"; (10) "Research on insecticides" (by ALBERT HARTZELL); and (11) "Fungicide investigations" (by S. E. A. MCCALLAN). In chapter 12 fourteen shorter miscellaneous projects, which in the main have been finished or discontinued, are described. Most of the problems described in the first eleven chapters have been under investigation throughout the life of the Institute, and some phases of all, except duck food and cell membranes, are still under study.

The research work reported in the book shows progress toward translating the ideals of the founder into practical procedures. The fact that a ten-million-dollar endowment was provided for research work to increase the provision for man's basic needs has social significance, and the problems solved contribute not only to agriculture and industry but also

to an understanding of the natural processes of living plants. Examples of this are: breaking the dormancy of the buds of potato tubers by the use of ethylene chlorhydrin, the storage of potato tubers under such conditions of temperature and carbon dioxide concentration as will prevent excessive formation of reducing sugars and thus prevent discoloration in potato chips made from them, the effects of chemicals in the ripening of fruits, causes of dormancy in seeds and methods of breaking the dormancy, the use of synthetic hormones to promote good rooting in cuttings, the control of color in the flowers of hydrangea by the use of aluminum, and the restoration of plants that furnish food for migratory ducks, geese, and brants by restoral of the locks which prevented sewage and sea water from coming into the waters which are the feeding grounds of migratory wild fowl.

If we accept the statement in the Introduction (quoted from R. K. DUNCAN) that "there is but one way of lifting man to a higher moral and spiritual plane, and that is by lifting to a higher plane the condition of his material surroundings," it is evident that the 20 years of research at the Institute has contributed considerably to carrying out the ideals of the founder.

That the plans of the director and his colleagues regarding the aims, organization, and scope of the work of the Institute have been followed and have worked well is evident from reading the book and the numerous contributions from the Institute which are cited. A summary at the end of each of seven of the chapters presents the essential facts and ideas in brief form. Of the one hundred and seventy-one figures, eight are full-page reproductions of color photographs, and many others are reproductions of black-and-white photographs. The other figures are graphs, diagrams, and maps. Many of the forty-eight tables are extensive, one of them covering two pages.—GEO. B. RIGG, *University of Washington, Seattle.*